PERCUTANEOUS ABSORPTION OF SELECTED HYDROPHILIC COMPOUNDS - AN *IN VITRO* STUDY ON HAIRLESS MOUSE SKIN

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INTRODUCTION - STATEMENT OF THE PROBLEM

It is only through a sound knowledge and understanding of the basic physical chemistry of the processes of drug delivery and skin permeation that it will be possible to optimize topical dosage forms. This very recent conclusion by leading scientists in the field of percutaneous absorption, reiterated the need for more systematic work on the basic physico-chemical aspects of percutaneous absorption (Hadgraft, 1983:52).

Studying the different layers of the skin, the nature of their biochemical and physiological activities and the interactions between barrier elements and penetrants, should indicate how to manipulate the structure of drugs and the pharmaceutical formulation to cause selective and effective permeability. With increased understanding of percutaneous absorption, the advent of singularly effective new topical drugs can be expected - drugs which are incorporated into precisely designed delivery systems which fully exploit the potentialities of the drug for treatment of specific disease states.

Much work has been done to elucidate skin structure, physiology, barrier properties and the mechanism by which substances enter and cross the skin. In the past two decades the scientific study of percutaneous absorption has moved from emphasising descriptive detail to the correlation of physico-chemical factors with skin permeation in attempts to understand which molecules may be expected to breach the skin (Hadgraft, 1983:52).

A major problem in the study of percutaneous absorption is the interpretation of results of previous investigators, since the permeability of mammalian skin has interested scientists from different disciplines. It is difficult and dangerous to draw conclusions and comparisons due to the use of various test systems, *in vitro* and *in vivo* models, various times and modes of application of material, and several species from mice to humans. The results have been expressed in many different ways and each researcher has been anxious to express the data so as to elucidate his or her own problem without making assumptions about the mechanism (Idson, 1975:902).

It is therefore essential to further the understanding of the processes of percutaneous absorption by the systematic characterization of the various factors involved in skin permeation.

The permeation of a membrane by chemicals is determined by the physico-chemical nature of the permeant, the physico-chemical nature of the medium of application and the physico-chemical nature, organization and the dimensions of the phases of the membrane. These factors influence the partitioning of the permeant between the solution phases external to the membrane and the phases of the membrane.

Partitioning was recognized as a factor in absorption by Meyer (1899:109) and Overton (1901:1). In recent years partitioning was even further generalized as an activity determining factor through

the empirical correlations of Hansch (1963:2817). Since positive correlations between hydrophobicity and the percutaneous absorption of many series of compounds have been reported, the permeability characteristics of skin have often been regarded as those of a simple lipoidal barrier (Valette et al., 1954:241; Treherne, 1956:171; Lien and Tong, 1973:371; Smith, 1982:158). A need existed for a study that would include compounds over a wide range of hydrophobicities to test the validity of such conclusions. If a direct relationship between hydrophobicity and percutaneous absorption should be found in such a study, it will support the view that the behaviour of skin approximates that of a simple lipoidal barrier. Otherwise the view that the skin behaves like a complex membrane where different permeation mechanisms for compounds of different hydrophobicities should be proposed, will be supported.

A base of data on the percutaneous absorption of a series of alkanols (Dürrheim et al., 1980:781) and hydrocortisone and its 21-n-alkyl esters (Smith, 1982:100) derived from in vitro work on hairless mouse skin already exists. Hydrocortisone and its 21-n-alkyl esters and the alkanols are two series of compounds that display relative little hydrophilicity. Therefore data on the percutaneous absorption of hydrophilic compounds would complement the existing data very well.

Therefore the first objective of this study was to inquire into the percutaneous absorption of selected hydrophilic compounds and the relationship between their hydrophobicity and percutaneous

absorption in order to supplement existing data. From the total base of data general conclusions may then be made about the relationship between hydrophobicity and percutaneous absorption.

The work of Treherne (1956:171) on the relationship between the ether-water partition coefficient and percutaneous absorption of a few polar nonelectrolytes has been cited much in the literature and was taken as a point of departure. He used radio-labelled ethyl iodide, methanol, ethanol, thiourea, glycerol, urea and glucose in percutaneous absorption studies on rabbit skin in vitro. Ethyl iodide was found to be unstable in the isotonic sodium chloride solution that was to be used in the diffusion cells and sufficient data on methanol and ethanol already existed. Therefore only urea, thiourea, glycerol and glucose were selected to provide a starting point in the systematic characterization of the percutaneous absorption of hydrophilic compounds.

Since Treherne (1956:171) used other techniques, diffusion cells and rabbit skin, his data did not fit into the existing data base, which was obtained using modern techniques, other diffusion cells and hairless mouse skin. Therefore percutaneous absorption studies of the selected hydrophilic compounds through hairless mouse skin had to be done in order to reach the goal of the study.

In the course of the permeability studies, a very slow but gradually accelerating permeation of the hydrophilic compounds was noted, a phenomenon that became very noticeable when the permeation profile was observed over longer periods, e.g. 100 hours instead of the conventional 6-12 hours.

It was therefore decided to do permeation studies with one of the hydrophilic compounds under various conditions to try and find the cause of the increasing permeability. Urea seemed to be a good choice because: reproducible results were obtained in the preliminary study with urea; the increase in permeability as a function of time was the most pronounced in the case of urea in the preliminary study; and urea is used in many preparations for application to the skin, so that its effects on skin are interesting in their own right.

The objectives of this study may thus be summarized as:

1. to contribute to a current data base in order to obtain a set of data on the ether-water partition coefficients and permeability coefficients of a variety of compounds ranging from very hydrophobic to very hydrophylic in order to assess

    - the relationship between hydrophobicity and percutaneous absorption; and
    - the validity of the view that the skin can be diffusionally characterized as a simple lipoidal barrier.

2. to systematically search for the cause of the increasing permeation as a function of time of urea in vitro through hairless mouse skin.

CHAPTER 1 PERCUTANEOUS ABSORPTION

1.1 INTRODUCTION

Until the turn of the century the intact skin of man was generally regarded as impermeable to all substances except possibly to gasses (Fleischer, 1877:81). By 1904 Schwenkenbacker and others had done enough systematic work in order to be able to generalize on the comparatively greater skin permeability of lipid-soluble substances and the relative impermeability of the skin to water and electrolytes. The fanciful idea of the complete impermeability of the skin was dispelled and a great deal of research has been done during the past 80 years in the area of percutaneous absorption. However, the research has been largely dominated by the dermatologist and biologist and neglected by the pharmacist and pharmacologist. Thus much work was clinically orientated and contained very little basic physico-chemical data of such a nature as to aid in the interpretation of the process of percutaneous absorption (Scheuplein and Blank, 1971:702).

Anyone who wants to do research in the field of percutaneous absorption has to assess the relevance of such research and must have a proper knowledge of the factors that influence percutaneous absorption and of the skin as a permeability barrier.

1.2 STRUCTURE OF THE SKIN AS A PERMEABILITY BARRIER

Only through the mechanistic understanding of the barrier properties of skin and particularly those of the stratum corneum will it become possible to adequately interpret experimental and

clinical results related to percutaneous absorption (Smith, 1982:25), a point reiterated by Hadgraft (1983:52).

Skin is an example of a structurally complex, thick membrane. Molecules moving from the environment across the intact skin of living man must first penetrate the stratum corneum and any material of endogenous or exogenous origin which may be on its surface, then the viable epidermis, the papillary dermis, the capillary walls into the blood stream, or into the lymph channels, whereupon they will be removed from the skin by the flow of blood or lymph. In order to move, molecules will have to overcome a different resistance in each tissue (Blank & Scheuplein, 1969:4). See figure 1-1 for a schematic cross section of the skin.

1.2.1 STRATUM CORNEUM

The stratum corneum is the outermost layer of the skin and part of the epidermis. It is composed of metabolically inactive, fully keratinized, flattened cells which are stratified in ten to thirty layers. The stratum corneum varies in thickness over the body and even at given anatomical sites between individuals (Elias et al., 1981:300). The flattened cells at the surface of the skin are continually shed as the forces holding these dead cells together progressively weaken towards the surface of the skin (Scheuplein and Blank, 1971:707).

The cell structure of the stratum corneum has much resistance to environmental insult, a characteristic which is provided by the thickened cell membranes and the amorphous cell matrix which contains keratin and other sulphur-rich proteins with many disulphide

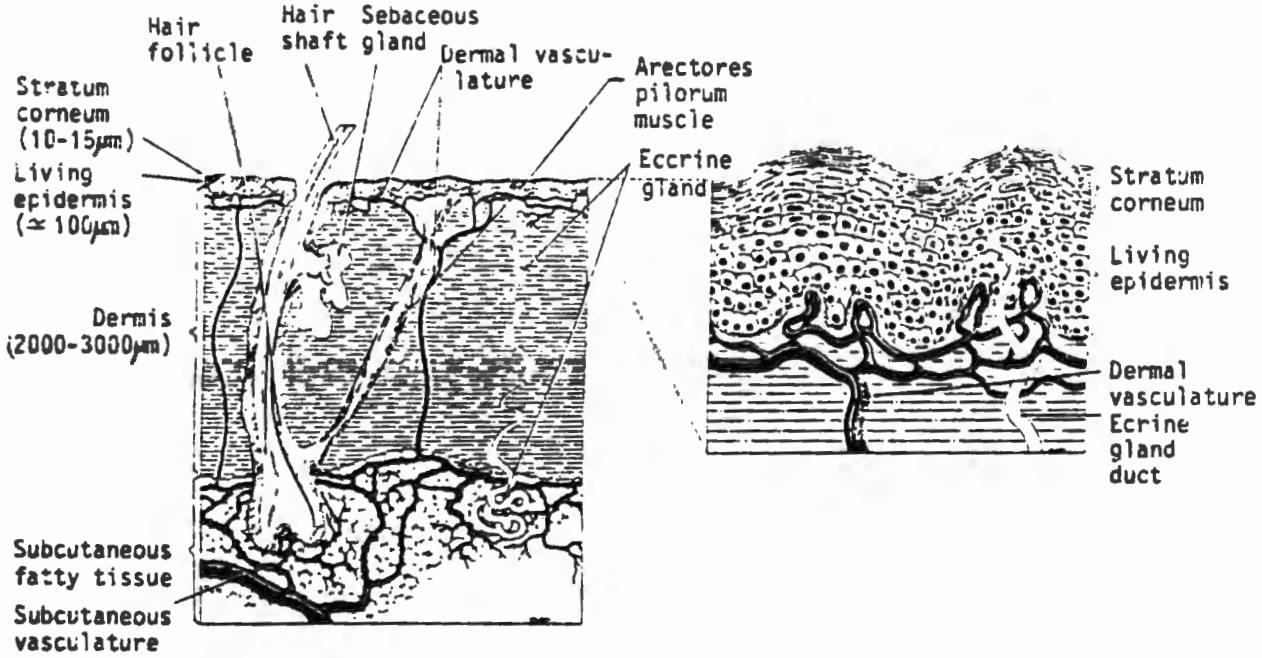

Figure 1-1 A schematic diagram of the cross section of skin (Flynn, 1979:267).

linkages and lipids (Mershon, 1975:44).

The ultrastructure of the stratum corneum is not fully known at this stage, although much progress has been made in this field in recent years. Very comprehensive reviews of the ultrastructural aspects of skin have been presented by Mershon (1975:41) and Smith (1982:36). The following aspects should be mentioned because of their possible relevance to the barrier properties of the stratum corneum.

The membranes of most cells are similar, being composed of proteins and fatty substances. Internal membranes are mainly phosopholipid, while cytoplasmic membranes contain large fractions of glycolipids and neutral lipids. Lipids account for about half of the mass of most cell membranes. The model of the arrangement of lipids in membranes is that of a bilayer about 50 angstroms thick with the hydrophobic chains orientated towards the membrane interior and the polar headgroups projected into the aqueous media of the cytoplasm and the external space (see figure 1-2)(Douglas & Zuckermann, 1976:23). It is suspected that a good deal of this organization is maintained in the stratum corneum but there are no definite studies which demonstrate this molecular organization. As a result of the highly specialized function of the stratum corneum, the membranes of the stratum corneum cells merge with the intercellular substance to form a possible hydrophobic medium for passive diffusion (Elias et al., 1979:339).

The arrangement and cellular structures of the stratum corneum show similarities in different species (Christophers, 1971:169). The cells have a columnar arrangement, are roughly hexagonal in

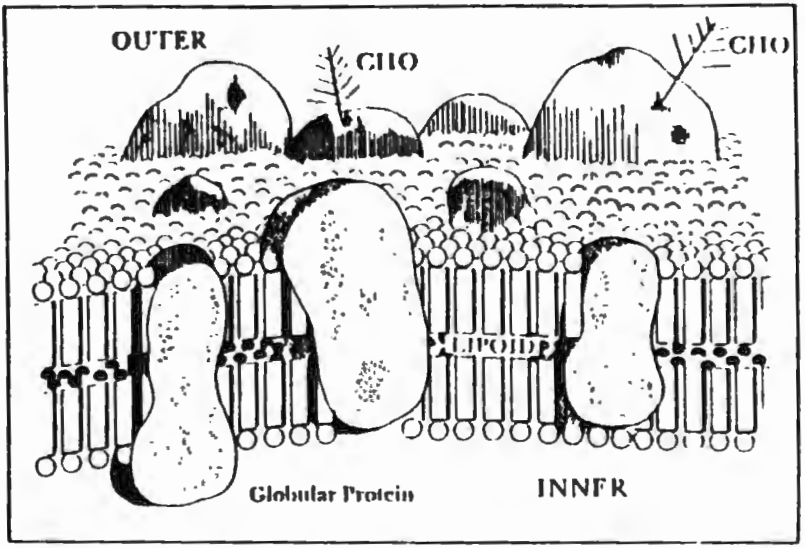

Figure 1-2 A modern model of asymmetrical fluid lipid-globular protein mosaic membrane (Douglas & Zuckermann, 1976:23).

shape and overlap slightly at the edges (Mershon, 1975:44).

The studies of Elias et al. (1981:297) indicate that lipid is deposited between and on the cells of the stratum corneum. That would explain the hydrophobic nature of the skin surface and the impermeability of the stratum corneum to hydrophilic substances (Treherne, 1956:178; Scheuplein & Blank, 1971:709).

There appear to be different water binding regions in the stratum corneum and the various polar regions on the surface and the inside of the proteinaceous fibrils composing the stratum corneum cell matrix may be hydrated to different extents and more polar regions may become accessible with continued hydration. Differential thermal analysis of the epidermal structures has indicated that three types of water in terms of the degree of binding are found in the stratum corneum (Van Duzee, 1975:406).

Elias & Friend (1975:180) found that water soluble tracers placed on either side of the stratum corneum of whole neonatal mouse skin would not penetrate the stratum corneum because of the presence of lamellar bodies in the intercellular space. The thickness of the intercellular space varied widely from place to place, but the dense zone containing these bodies formed an uninterrupted sheet of 16 to 20 nm in thickness. This supports the earlier speculation of Brody (1966:472) that the variable penetration rates of chemicals and solvents through the skin might be due to the varying amounts of this intercellular component.

Although no two investigators present exactly the same picture on the ultrastructure of the stratum corneum, there are indications that polar and nonpolar regions exist in the stratum cor-

neum. These likely have very different densities and therefore molecular diffusion can be expected to be very different between domains. From the apparent nature of the cell matrix, a wide range of partitioning dependencies might be expected, depending on the nature of the permeating substance. Thus, accessability and mobility within various regions in the stratum corneum may be dependent on molecular size and molecular formula. A very polar molecule may selectively partition primarily into a polar region, a nonpolar molecule into a very hydrophobic region but the diffusional relationship between the skin and a simple lipoidal barrier has not been systematically assessed. Small polar molecules (like some of the selected hydrophilic substances in this study) will not easily diffuse through the lipid rich intercellular spaces but will more readily partition into the polar regions provided by the polar headgroups of the bilayer cell membrane structures. Here they may be soluble but possibly also highly immobile, limiting the diffusion through the protein-rich domain. The hydrophobic inner part of the bilayer largely prevent the polar molecules from entering the intracellular area. That leaves a very restricted area for diffusion of the polar molecules, which might explain why these molecules have low diffusivities in the stratum corneum. Scheuplein & Blank (1971:719) found that the average, apparent diffusion coefficient for the nonpolar alcohols and water in the stratum corneum is approximately constant at a value of 5×10^{-10} cm^2/sec and concluded that this value may be considered typical of the skin diffusivity of many simple nonelectrolytes through hydrated skin.

1.2.2 VIABLE EPIDERMIS

The stratum germinativum, the innermost layer of the epidermis, is a single mitotic layer of basal cells which divide constantly to produce a gradual outer displacement of cells towards the surface of the skin. While in residence in the stratum germinativum the cells produce strands of protein which are woven into a proteinaceous end product called keratin during the movement of these cells toward the skin surface (Smith 1982:28). The cells exchange their columnar appearance for a more rounded appearance in the stratum spinosum or prickle cell layer. Enzymes are present in this layer and cell respiration and metabolism are very active (Smith 1982:46). The stratum granulosum is about 12 to 15 layers above the stratum germinativum and consists of more flattened cells with a granular appearance. These cells are considered to be in the last stages of viable existance (Flynn, 1979:269).

All of the above layers constitute the Malpighian layer of the epidermis or the layer of living, metabolitically active cells. This is often referred to in total as simply the viable epidermis. The viable epidermis is primarily an aqueous solution of proteins encapsulated into cellular compartments by their cell membranes. The cells are tied together by tonofibrils. The density of the cell contents is close to one, thus very similar to that of water (Flynn, 1979:269).

The self-diffusion of water is about ten times greater than the diffusion of water through the cellular mass of the epidermis. By means of experiments where the stratum corneum was stripped to different extents, it became clear that the viable epidermis is

an inferior barrier to the penetration of all compounds (Flynn et al., 1981:52). One would thus expect a diffusion coefficient of about 1×10^{-6} cm^2/sec for the small polar nonelectrolytes in the viable epidermis. The viable epidermis is thus not regarded as a rate-determining barrier in percutaneous absorption through full thickness skin.

1.2.3 DERMIS

The dermis is a much thicker layer than the epidermis. The thickness varies from 1 to 5 mm, which is 5 to 30 times the thickness of the epidermis (Smith, 1982:31). The surface of the dermis contains pappillae which contain capillary loops and nerve endings and help to form a firm adhesion between the dermis and epidermis. The dermis has a rich blood supply, it contains lymphatic channels and is interrupted by appendages of epidermal origin (hair follicles, nail plate and sweat glands). It also contains the reticulo-endothelial system which plays a significant role in inflammation and disease. The base of the dermis joins the subcutaneous fatty region. The dermis is a dense network of structural protein fibers (collagen, retikulum, elastin) embedded in a semigel matrix of mucopolisaccharide ground substances (Flynn, 1979:270).

The dermis has been diffusionally characterized as being functionally an aqueous gel with no significant amount of cellular structure or lipid content. It contains up to 70 per cent water and offers little more than the diffusional resistance of an equivalent thickness of water (Dürrheim et al., 1980:784). Generally, if a substance penetrates the stratum corneum and the viable

epidermis into the dermis and hypodermis, it is readily absorbed into the circulation.

1.2.4 THE APPENDAGES

Hair follicles, sebaceous glands, nails, eccrine and apocrine glands are skin appendages of epidermal origin which originate in or beneath the dermis and extend to the surface of the skin and thus form channels through the skin which circumvent passage through the stratum corneum. Usually only the hair follicles are regarded as a possible significant diffusional pathway, especially for the pre-steady state permeation of hydrophobic molecules like the steroids (Scheuplein et al., 1969:63). Very recently Scheuplein has presented results suggesting that for highly polar substances the shunt mechanism was important (Hadgraft, 1983:52).

1.3 FACTORS INFLUENCING PERCUTANEOUS ABSORPTION

Percutaneous absorption involves the movement of molecules across epidermal cellular structures. Therefore the factors that influence percutaneous absorption are essentially the same as those that influence gastro-intestinal absorption. Additional variables are the condition of the skin, the skin age, the area of skin, the thickness of the barrier phase, species variation and the moisture content of the skin. The primary factors that determine the rate of diffusion through the skin are the physico-chemical properties of the drug. Secondary factors are the nature of the

vehicle, the pH, the concentration of the drug and temperature (Idson, 1975:909).

Excellent reviews of the differend factors affecting percutaneous absorption can be found in recent literature (Katz and Poulson, 1971:117; Idson, 1975:909; Dürrheim, 1977:40). Not all the factors will be discussed here but since the hydrophobicity of the permeating compound and the hydration of the skin are impor= tant factors in this particular study, they will be discussed briefly in the subsequent sections.

1.3.1 HYDROPHOBICITY

The solubility characteristics of a substance greatly influence its ability to penetrate biological membranes. The partition coefficient of a compound has long been recognized as having some definite relation to its permeation through skin (Valette et al., 1954:241; Treherne, 1956:171; Katz and Shaikh, 1965:291; Lien and Tong, 1973:371).

Realistically the only partition coefficient measurement for a penetrant that can be exactly related to its rate of diffusion through the skin is the value determined for the equilibrium distribution of the penetrant between the vehicle and the stratum corneum. However, even experimental values determined between stratum corneum and water are not that reliable. Since partition coefficients are equilibrium values, appreciable periods of time may be required in laboratory experiments where the penetrant is distributed between strips of stratum corneum and the bathing fluid. It the bathing fluid is water, the stratum corneum may

achieve an abnormally high degree of hydration. It must also be kept in mind that experimentally determined partition coefficients will reflect not only the relative solubility of the penetrant in the stratum corneum but also interactions between penetrant and skin components such as physical binding or complexation (Katz and Poulson, 1971:137). These are some of the reasons why many researchers chose other lipophilic solvents to simulate the lypophilicity of the stratum corneum in partition coefficient/ permeability correlations.

Unfortunately the unique, encompassing solubility characteristics of the stratum corneum could not be duplicated over the full range of polarity by any lipophilic solvent. For this reason previous attempts to predict permeability coefficients, generally from limited correlations found with olive oil-water or ether-water partition coefficients, have proven unsuccessful (Treherne, 1956:171; Cronin and Stoughton, 1963:81; Scheuplein et al., 1969:63).

However, no systematic study of the correlation between ether-water partition coefficients and percutaneous absorption of substances over the full range of polarity has been conducted so far. Such a stucy may lead to significant structuring of data and contribute to the overall objective of all percutaneous absorption studies, namely to predict the permeability of structurally unrelated compounds from their physico-chemical properties only (Ando et al., 1983:1).

1.3.2 HYDRATION

Hydration of the stratum corneum results from water diffusing from underlying epidermal layers or from perspiration accumulating after application of an occlusive vehicle or covering on the surface. Under occlusive conditions, the stratum corneum is changed from a tissue that normally contains very little water (5-15 per cent) to one that may contain as much as 50 per cent water (Idson, 1975:910). The water-binding properties of the stratum corneum appear to result from the presence of certain hygroscopic substances in the horny layer (Smeenk and Rijnbeek, 1969:476).

When the stratum corneum is immersed in water *in vitro*, it is not immediately hydrated. Although maceration may be detected in a few minutes, swelling continues for fully three days. The tissue can absorb 5-6 times its weight when fully hydrated and this water is strongly bound within the intracellular keratin (Scheuplein, 1966:1). The filament-matrix ultrastructure is preserved under hydration and the water appears first to enter between the filaments and only later to diffuse within them. The separation between the lipid and protein components of the tissue is probably increased on hydration since the water would tend to build up irregular multilayers on the fibrous protein and help screen it from the lipid. At the macromolecular level the intracellular keratin appears to be a stable, continuous, water-rich polar region intermingled with a network of nonpolar lipid.

Hydration of the stratum corneum is among the most important factors in skin penetration because it appears to increase the rate of permeation of all substances that penetrate the skin

(Idson, 1975:910). Hydration of the epidermis has been shown to increase the percutaneous absorption of nicotinic acid (Cronin and Stoughton, 1962:265), esters of salicylic acid (Wurster and Kramer, 1961:288), steroids (McKenzie and Stoughton, 1962:608) and alkanols (Behl et al., 1980:346). These compounds were affected to different degrees by hydration - as little as a two-fold increase in permeability of butanol and hexanol to a hundred-fold increase in the permeation of corticosteroids. Scheuplein and Blank (1971:719) however, have shown that even hydrated stratum corneum is still an enormously effective diffusion barrier with a diffusional resistance almost 10 000 times greater than an equivalent layer of water.

One would expect the rate of penetration of water-soluble compounds to be faster through hydrated than non-hydrated stratum corneum, but the low diffusion coefficients and high activation energy obtained for water and polar alcohols and the selective diffusion exhibited by molecules of varying polar character suggest that extensive hydration does not drastically affect the barrier function of the stratum corneum (Scheuplein and Blank, 1971:719).

The work of Wurster and Kramer (1961:588) and Yotsuanagi and Higuchi (1972:934) showed that the permeability behaviour of fully hydrated stratum corneum showed a reasonable correlation between experimental permeability coefficients and lipid-water partition coefficients.

In a study of the influence of hydration on alkanol permeation, the same techniques and hairless mouse skin as in this study were used. It was found that hydration with normal saline for up to

30 hours did not significantly affect the permeabilities of water, methanol and ethanol (Behl et al., 1980:346). Though the effect of hydration on the percutaneous absorption of other polar non-electrolytes has not been studied systematically, one would not expect a great hydration effect because of the very similar penetration characteristics (Scheuplein and Blank, 1971:716) of water and other polar nonelectrolytes.

1.4 CONCLUSIONS

The ultrastructure of skin is not adequately known at this stage to enable predictions about the molecular interactions of the skin with penetrating substances. On a macroscopic level however, different strata with different structures and resistances to diffusion can be distinguised. Solubility and permeability data obtained in the past support the postulate that polar and non-polar solutes diffuse through the stratum corneum by different molecular mechanisms. The membrane partition coefficients for highly water soluble compounds are all near unity, suggesting that they dissolve into hydrated regions of the keratin. Entering water molecules and other highly polar molecules would therefore tend to diffuse within these aqueous regions, located probably near the outer surface of the filaments rather than deep within their semicrystalline interiors. In contrast, non-polar lipid-soluble molecules would tend to dissolve and diffuse within the lipid network between the filaments. A common feature of both mechanisms is the substantially higher free energy of activation for diffusion within the stratum corneum than for the same molecules in solution. This is the underlying reason why

diffusion for all solutes occurs so slowly and implicates a strong solute-tissue interaction and a semi-solid structure for the stratum corneum. The topicality of knowledge of the physico-chemical properties of the permeant, permeant/skin interactions and the ultrastructure of the skin and particularly the stratum corneum as a means to explain the permeability behaviour of skin has been emphasized by leading scientists in the recent past (Elias et al., 1981:297; Briggaman, 1982:1; Grayson and Elias, 1982:128; Smith et al., 1982:7; Hadgraft, 1983:52). It is clear from the literature search that much information on these aspects is still needed for the optimization of topically applied preparations. The correlation of the physico-chemical properties of the permeant with its percutaneous absorption is still very relevant in this field and it will stay relevant until the ideal biophysical model has been developed.

Virtually all *in vitro* methods include the use of some type of diffusion cell in which animal or human skin is fastened to a holder and the permeation from the epidermal surface to a fluid bath is measured. The percutaneous absorption can be quantitatively measured and the simplicity of the equipment has prompted widespread use of *in vitro* techniques. The disadvantage of *in vitro* techniques is that it does not reflect the role of the skin *in vivo*. But the passive nature of skin permeability is entirely consistent with the location of the major diffusion barrier in the stratum corneum, which consists of keratinized metabolically inactive cells. No active transport process across the skin has been demonstrated so far. Thus the usual diffusion laws of physics pertaining to passive diffusion processes can therefore be applied to skin permeability phenomena and greatly aid in its description (Scheuplein, 1971:706). The large amount of *in vitro* research on skin permeability testifies to the widespread acceptance of this view. This view is supported by solid experimental results (Ainsworth, 1960:69; Blank *et al.*, 1967:582; Scheuplein *et al.*, 1969:63; Dürrheim *et al.*, 1980:781).

2.2.2 HUMAN SKIN

Human skin would seem to be the natural choice for any study of percutaneous absorption aimed at defining the properties of the stratum corneum in terms of clinical or therapeutic situations. However, there are several problems associated with the use of human skin in an *in vitro* experimental situation. Sources are not reliable in that they are subject to the availability of

2.2 EXPERIMENTAL METHODS AND THE RELEVANCE OF HAIRLESS MOUSE SKIN AS A MODEL FOR PERCUTANEOUS ABSORPTION

2.2.1 *IN VITRO* VERSUS *IN VIVO* TECHNIQUES

The experimental methods can be divided into *in vitro* and *in vivo* procedures involving two general types of test systems. The *in vitro* methods involve excised skin in diffusion chambers and the *in vivo* methods employ the skins of the living animal or human subject *in situ*.

It is generally accepted that *in vitro* data cannot be directly extrapolated to *in vivo* or clinical situations and that *in vitro* and *in vivo* data should rather complement each other (Blank & Scheuplein, 1969:4; Marzulli *et al.*, 1969:76). Both *in vivo* and *in vitro* measurements for a given agent are highly desirable (Marzulli *et al.*, 1969:82). Correlations between *in vivo* and *in vitro* percutaneous absorption have been observed for water (Baker & Kligman, 1967:450), ions (Spruit & Malten, 1968:11) and non-electrolytes (Scheuplein *et al.*, 1969:63). The major *in vivo* methods involve histological studies (Calman, 1970:26), use of tracers (Malkinson and Ferguson, 1955:281), elicitation of biological responses after topical application (Stoughton, 1969:753) and analysis of body fluids or tissues (Ackermann & Dreyer, 1982:28). The main objection to the use of the living animal or human subjects is that this method measures percutaneous absorption by indirect means and results are difficult to quantify (Idson, 1975:902).

CHAPTER 2 METHODS AND PROCEDURES FOR MEASURING AND ANALYSING PERCUTANEOUS ABSORPTION

2.1 INTRODUCTION

During the past decade or two the basic concepts of membrane transport have been applied specifically to transport through the skin. Better techniques have been developed and more sophisticated experiments have been designed. Much of the recent data has been obtained from experiments on excised skin under laboratory conditions, because of the difficulty in obtaining quantitative *in vivo* data.

Numerous methods for studying percutaneous absorption have appeared (Treherne, 1956:171; Cronin & Stoughton, 1963:81; Halprin & Ohkawara, 1967:561; Marzulli *et al.*, 1969:76; Scheuplein & Blank, 1973:286; Foreman *et al.*, 1977:108; Schaeffer *et al.*, 1978:80; Epstein *et al.*, 1979:207; Dürrheim *et al.*, 1980:781; Nemanic & Elias, 1980:573; Wedig & Maibach, 1981:433; Wohlrab & Hassler, 1981:277; Pitman & Downes, 1982:63). No attempt will be made to cataloque the extensive literature but rather to motivate the use of certain methods and procedures in this study.

The methods and procedures for measuring and analysing percutaneous absorption include the experimental techniques, the mathematical analysis of permeability data and modelistic approaches. These aspects are usually employed in conjunction with each other in percutaneous absorption studies in order to contribute towards the improvement of techniques and the refinement of mathematical models.

cadaver donors. Thus it is not available on demand and must be stored frozen for variable periods (Scheuplein, 1965:335), a condition that may induce physico-chemical alterations of the skin. Preparation of excised human skin membranes for permeability studies is relatively complicated. Much of the underlying fatty tissue must be removed and it requires special equipment and skill to prepare a membrane of uniform thickness and free from holes and other defects (Katz & Poulson, 1971:103).

Thus human skin is not procured as easily as an animal membrane and its properties vary from sample to sample due to differences in the age, sex, race and health of the donor, even if it is removed from the same anatomical site (Tregear, 1966:44).

2.2.3 ANIMAL SKIN

Since *in vivo* studies on human subjects are costly and time-consuming due to the necessary safeguards, the only alternative left to either of these approaches is the *in vitro* studies with animal skin. An animal membrane that is easily obtainable and relatively invariant from animal to animal could simplify the process of screening topical drugs. But no animal skin sufficiently resembles human skin to serve as a general model (Montagna, 1971:577).

However, certain animal skins may resemble human skin in certain properties and may be suitable for a particular type of study. In terms of anatomical features that contribute to the unique barrier properties of human skin, such as sparse hair distribution and a thickened epidermis with a highly cornified stratum

corneum, the skins of essentially nude mammalian species have been suggested as suitable approximations. Of the pig, minipig and hairless mouse, only the hairless mouse is sufficiently small and inexpensive to make animal sacrifice for individual experiments practical. For the reasons of availability and convenience, the past decade has seen a dramatic increase in the use of nude and hairless mice in dermatological research (Jain & Wray, 1978:1294).

Hairless mice are easy to keep under strict environmental and nutritional conditions and are always available for experimentation. Damaged skin can be avoided by selectively rejecting any diseased or mutilated animals. Animals of one sex and age range may be chosen. Another important experimentally exploitable feature of the hairless mouse is that the skin over both the dorsal and abdominal surfaces is loose and nonadhering to the viscera, making it possible to remove skin membranes of uniform thickness without recourse to microtomy (Dürrheim et al., 1980:784). Thus the hairless mouse has distinct advantages as an experimental animal for percutaneous absorption studies in vitro.

The skin is histologically similar to that of the human, but lacks the fatty subcutaneous layer at the base of the dermis. Hairless mouse skin has a stratum corneum which is one third thinner than that of human skin (Smith, 1982:25). Stoughton (1969:753) was able to show that penetration rates for several anti-inflammatory steroids through human and hairless mouse skin were much the same. Hairless mouse skin has also been shown to be similar to human skin in its permeability to the homologous n-alkanols (Dürrheim et al., 1980:781; Flynn et al., 1981:52). Though similar results for hairless mouse skin and human skin have

been obtained for certain compounds, it does not guarantee that permeability parallels will persist over the full range of compounds of interest. Therefore constant care must be taken in applying conclusions to human skin drawn from data derived from animal experimentation.

In vitro measurements of percutaneous absorption are invariably measurements of steady-state rates because they are the most readily obtainable in the laboratory situation. Steady-state forms of the diffusion laws are therefore of major importance and interpretations of permeability phenomena in terms of steady-state parameters are major concerns (Scheuplein & blank, 1971:706).

2.3 MATHEMATICAL ANALYSIS OF PERMEABILITY DATA

Any given membrane is composed of distinct macroscopic phases and the passive diffusive current across the total membrane barrier will be dependent on the organization and sequencing of all encountered phases in the transport process. The process will be dependent on the physico-chemical natures of the phases and the interaction between the permeant and the respective phases. The densities and viscosities of the phases affect the rate of diffusive movement of the molecules, while the permeant-membrane interactions are reflected in solubilities and partition coefficients (Smith, 1982:48).

All phases in a membrane may be characterized in terms of a diffusional resistance. The phases can be arranged in series or in parallel, or they may appear as some form of dispersion in the

diffusional field. Fick (1855:59) recognized this analogy and formulated a set of diffusion equations by direct analogy with the equations of heat conduction. The integral form of Fick's diffusion law, which strictly applies to a simple homogenous membrane only in the absence of complicating influences as caused by hydrodynamic layers, is expressed in equation 1 (Scheuplein & Blank, 1971:713).

$$J = \frac{D(C_1 - C_2)}{h} \qquad \text{..... Equation 1}$$

where J = Steady-state flux of the solute (moles cm^{-2} hr^{-1})

D = Average membrane diffusion coefficient for the solute (cm^2 sec^{-1})

$(C_1 - C_2)$ = ΔC = Concentration difference of solute across membrane (moles cm^{-3})

h = Membrane thickness (cm)

The diffusional resistance of a phase can be explicitly defined in terms of the thickness of the phase, h, the permeant diffusion coefficient in the phase, D, and the partition coefficient, K, which is equal to the concentration in the membrane phase divide by the concentration in the external phase. The partition coefficient is brought into the equation because the stratum corneum is not simply an inert material, but one with an affinity for the applied solute. Thus the concentrations at the surfaces of the membrane are not usually equal to the concentrations in the external solutions, but are related to them in accord with the sorption isotherm which is often linear in the dilute concentration range according to Henry's law (Scheuplein & Blank, 1971:713).

Equation 1 then becomes

$$J = \frac{K\,D\,\Delta C}{h} \qquad \text{..... Equation 2}$$

According to equation 1 the steady-state flux per unit area, J, should be proportional to the concentration difference, ΔC. The proportionality factor is the mass transfer coefficient or permeability coefficient, P, and is:

$$P = \frac{K\,D}{h} \qquad \text{..... Equation 3}$$

The individual permeability coefficient for an isolated phase, whether in series or parallel or otherwise is the reciprocal of the diffusional resistance of that phase (Flynn et al., 1974:479).

Membrane characterization is a matter of systematically and selectively altering either phase thicknesses, diffusivities or phase affinities. This can be done by physico-chemical manipulation with resulting homologue partitioning effects and permeant structural effects on solubility and diffusivity, variation of membrane or phase thickness, pH effects, techniques involving inhibition of membrane enzymes and solvent treatment of membranes (Smith, 1982:52).

The membrane-external phase partition coefficient, the diffusivity in the membrane and even the thickness of the membrane in some instances must be obtained by indirect means from other experimental data. Aguiar and Weiner (1969:210) derived equations for the permeability coefficient which contains experimentally measurable components.

In this study the steady-state phase of percutaneous absorption will be observed and analysed for which the abovementioned equations are valid. Thus the equations used to analyse the other data in the data base will also be used in this study - see 4.3.2.4 for the equations used in this study.

2.4 MODELISTIC APPROACHES

The percutaneous absorption of a compound depends on a complex series of processes involving parallel and sequential events which move the molecules from the point of application to the sites of biochemical or physical modulation of cellular activity and which at the same time remove the molecules from participation in the biological processes via excretion or metabolic alteration. Every step in this cascade, whether biochemical, chemical or physical, has structural dependency. The overall structure-activity relationship is a composite of all the contributing factors, including structure-permeation dependencies.

The lipophilicity of a permeating substance has long been recognized as an important factor in determining membrane permeability and has served as the basis of various modelistic approaches in percutaneous absorption (Treherne, 1956:178; Scheuplein and Blank, 1971:718). Yet correlation between lipophilicity and permeation rate need not always be observed as demonstrated in studies on tape stripped skin (DÜrrheim et al., 1980:783) and human (Scheuplein et al., 1969:63) and animal dermis (Dürrheim et al., 1980:783).

Where permeability relationships fit into the structure-activity picture has remained an elusive aspect to define. Different approaches to structure-activity relationships have been discussed by Smith (1982:9). It is clear from his discussion that the Free and Wilson (1964:395) and Hansch (1963:2817) approaches do not present realistic biological activity models, at least when it comes to mass transfer processes. The model presented by Higuchi & Davis (1970:376) is more representative of an actual biological situation, but is also not applicable to any situation where structure-activity depends on kinetic events related to structure, because it is premised on the development of a quasi-steady state between the drug concentration at or near the receptor and the concentration in the central compartment of the body (blood). Thus for the most part, the Higuchi & Davis model is inappropriate when it comes to considerations of percutaneous absorption.

It seems as if only a model which takes into account every small detail of every event during the permeation of a substance through the skin, will satisfy all the criteria for the ideal model. It is not at present possible to cope with a totally comprehensive model as individual contributing factors are as yet themselves not fully understood. Therefore, the useful approach at this time should involve isolation and exhaustive characterization of single events in the overall mechanism of percutaneous absorption. Many scientists have taken this road and studied some of the physicochemical laws affecting mass transfer across membranes (Scheuplein & Blank, 1971:702; Flynn & Yalkowsky, 1972:838; Dürrheim et al., 1980:781; Flynn et al., 1981:52).

With regard to the above, the following published works seem especially relevant. Yotsuyanagi and Higuchi (1972:934) examined

a two-phase series model for the permeability behaviour of the fully-hydrated stratum corneum using data on steroids. The two phases represented the cytoplasm and cell walls respectively. In general, reasonable self-consistencies among the various experimental results and parameters of the model were found. But the absence of independent values for the partition coefficients of the two phases precludes a rigorous test of the theory. Stoughton et al. (1960:337) examined a series of nicotinic acid esters and correlated absorption with the oil-water partition coefficients of the molecules. Similar studies were carried out by Treherne (1956:171), Katz & Shaikh (1965:591), Roberts et al. (1978:677) and Dürrheim et al. (1980:781) for a series of non-electrolytes, corticosteroids, phenols and alcohols respectively. Lien & Tong (1973:371) attempted to use multiple regression analysis to correlate absorption with steric factors, molar refraction, Taft's polar substituent constant and molecular weight. This approach gives the appearance of being able to relate the physico-chemical attributes of compounds to their absorption and is presented as being predictive. Unfortunately the correlations are trivial as the premise of the analysis is totally in error.

Scheuplein & Blank (1973:286) studied the permeation rates of a homologous series of primary alcohols (C_1-C_{10}) through human skin in vitro. The alcohols were applied from aqueous solutions and also as the pure liquids. They found that Fick's law holds as an approximation for both the aqueous and the liquid alcohol systems. They showed that with a knowledge of the solubility of the solute in the vehicle and in the tissue, they could predict the rate of percutaneous absorption of this series of compounds. These workers were also first to note the remarkable temperature sensitivity associated with permeation of human epidermal sec-

tions. Activation energies up to and exceeding 15 kcal/mole were recorded for the n-alkanols, which are much greater than for diffusion through a liquid-like phase, suggesting marked thermal disruption of the fine structure of the stratum corneum, possibly by 'melting' of its proteolipid organization. The effect of temperature on the permeation of phenolic compounds from aqueous solution through excised human skin has been examined by Roberts et al. (1978:486). From a thermodynamic analysis of the data, they postulated a mechanism by which these solutes penetrate through human skin. Diffusion of the more polar compounds through the stratum corneum appeared to depend on the breaking of hydrogen bonds in the desolvation of the solute and by the overall "viscosity" of the stratum corneum. The lipid barriers in the stratum corneum seemed to form the main resistance to the penetration of these compounds. The aqueous boundary layers appear to provide an additional barrier to the penetration of the non-polar phenolic compounds. Michaels et al. (1975:985) have described a percutaneous absorption model in which the stratum corneum is conceived of as being proteinaceous bricks held together by interstitial lipid mortar. But since it is extremely difficult to unambiguously discriminate structural pathways, this kind of modelistic approach seems to be premature and inappropriate at the moment.

A theoretical description of percutaneous absorption which included interfacial barriers, allowed for the depletion of the substance in the external phase and considered the transcellular and intercellular routes, has been derived by Albery and Hadgraft (1979:129). Since they had to use an idealized geometry for the cell structures involved, no change in the route of penetration with time could be indicated. This is in contrast with the possible change in mechanism because of initial follicular permeation

as discussed by Scheuplein & Blank (1971:705). The mathematical problem of simultaneous transport and metabolism in the skin and degradation in the receptor phase was solved by Loftsson (1982: 17). He found that his model was in good agreement with experimental data obtained for simultaneous diffusion and hydrolysis of acetylsalicylic acid in excised hairless mouse skin. Ando et al. (1983:28) developed a physical model where only the physical properties of the components are accounted for. The parameters that have been chosen are open to future verification and are only meant to represent the current state of knowledge.

These are but a few examples of studies with a modelistic approach. In most of these studies, however, the correlation between the model and experimental data had to be limited to a series of closely related compounds. The ideal would be to develop a model that can be used to predict human percutaneous absorption of any molecule from only its physico-chemical properties.

2.5 CONCLUSIONS

Percutaneous absorption can be studied in many ways, but the various techniques may not give comparable quantitative data. With present technique and their stated range of application it has not been possible to determine the avenues of penetration and rarely has the ultimate deposition of the penetrating substance been made clear. Progress in the area of percutaneous absorption is still being retarded by the absence of good methodology. The literature allows one to conclude that:

1. *in vivo* percutaneous absorption studies in man present many problems of which the most important is the difficulty of obtaining quantitative data. The main problems with *in vitro* studies on excised human skin are the preparation of the skin and the reproduceability of results. It seems therefore that mechanism studies and studies on parameters affecting absorption must be explored, using animals and *in vitro* techniques. For compounds studied to date the permeability of hairless mouse skin seems to tightly parallel that of human skin, seemingly making it ideally suited for *in vitro* studies;

2. the advantage of *in vitro* procedures is the absolute control one has over the environment, allowing the demonstration of the importance of individual factors in determining percutaneous absorption of a particular substance. These *in vitro* results may point the way for further *in vivo* work. If the outcomes with *in vitro* permeation studies remain generally consistent with all other experimental findings, then they may have a useful place in the screening of new products without the difficulties usually involved in *in vivo* methods;

3. it seems clear that the basic principles of Fick's law are applicable in almost all instances of percutaneous absorption; and

4. most of the modelistic approaches evolved from partitioning or solubility characteristics of the penetrants. Recent multiphasic concepts and compensation for metabolism and degradation have served as refinements to the older models.

CHAPTER 3 PHYSICO-CHEMICAL PROPERTIES AND TRANSMEMBRANE DIFFUSION OF SELECTED HYDROPHILIC COMPOUNDS

3.1 INTRODUCTION

Percutaneous absorption is considered as a process where the physico-chemical nature of the permeant, of the medium of application and of the phases of the membrane play vital roles in the overall description of the process. Knowledge of the physico-chemical properties of the selected substances is therefore essential for the interpretation of experimental results and to test the validity of conclusions.

A few hydrophilic compounds were selected to provide a starting point in the systematic characterization of percutaneous absorption of hydrophilic compounds. The work of Treherne (1956:171) on a few polar nonelectrolytes has been cited much in the literature and was taken as a point of departure. He used the following compounds in his percutaneous absorption studies on rabbit skin: Ethyl iodide, methanol, ethanol, thiourea, glycerol, urea and glucose.

Ethyl iodide was found to be unstable in the isotonic sodium chloride solution that was going to be used in the diffusion cells. Ethyl iodide forms in the presence of chloride ions, ethyl chloride which is a gas with a boiling point of 12,5^{o}C (Finar, 1967:135). This may be the reason why Treherne (1956: 171) reported very little data on ethyl iodide. (Treherne used Ringer solution in his receptor diffusion cells.) This problem made it impossible to work with radiotracer quantities of ethyl

iodide. Much work has already been done on the percutaneous absorption of methanol and ethanol through human skin (Scheuplein, 1965:334) and hairless mouse skin (Dürrheim et al., 1980:781; Flynn et al., 1981:52) and therefore data for these two polar alkanols were already available for the analysis.

The compounds selected for this study were initially limited to urea, thiourea, glycerol and glucose. The physico-chemical properties of these compounds will be listed in this section but the current knowledge on the diffusion of these compounds through membranes will be emphasised.

3.2 UREA

3.2.1 DESCRIPTION AND STABILITY

Synonyms for urea are carbamide, ureum and Harnstoff (German). The molecular structure is $NH_2.CO.NH_2$ and the molecular weight is 60,06. Urea comes in the form of colourless, slightly hygroscopic, odourless or almost odourless prismatic crystals, with a cooling saline taste. The melting point is 132°C to 134°C.

The solubility of urea is approximately 1 in 1 of water and 1 in 12 in ethanol. It is insoluble in chloroform and ether. Solutions in water are neutral to lithmus. A 1,63 per cent (w/v) solution is iso-osmotic with serum (Martindale, 1977:571).

The degree of degradation of 2M, 4M, 6M and 8M solutions of urea

at 25°C, 35°C and 45°C was extremely small and the overall process conforms to a first and second order reversible reaction. Urea in aqueous solution will slowly hydrolyse into carbon dioxide and ammonia (Welles et al., 1971:1212). Urea is incompatible with nitric acid, nitrites, alkalis and formaldehyde.

3.2.2 DIFFUSION THROUGH MEMBRANES

Urea has been used as an active ingredient in many commercial preparations designed for normal or pathological skin (Hellgren & Larson, 1974:289) and is considered to be part of the natural moisturization factor in skin. Different effects of urea on the skin have been reported in the literature e.g. an increase of the water binding capacity of the skin (Kligman, 1957:157; Swanbeck, 1968:123; Ashton et al., 1971:194; Hindson, 1971:284; Jacobi, 1971:108; Grice et al., 1973:114; Hellgren & Larson, 1974:289),

thinning of the epidermis (Wohlrab et al., 1974;378; Wohlrab & Böhm, 1975:150; Wohlrab, 1976:585),

keratolysis (Swanbeck, 1968:123; Hindson, 1971:284; Roston, 1971: 142; Vleeschouwer & Bersaques, 1971:225; Hellgren & Larson, 1974:289),

antibacterial activity (Kligman, 1957:157; Swanbeck & Rajka, 1970:226; Ashton et al., 1971:195) and

proteolytic activity (ibid).

In some clinically orientated experiments Feldman and Maibach (1970:399) detected 5,9 per cent of the applied dose of urea over a period of 5 days in the urine of humans. Wahlberg and Swanbeck (1973:209) also found a very low permeability for the permeation of urea through human skin. Experiments on the penetration of urea through hairless white minipig skin were conducted in vivo. The urea penetrated the skin very fast and could be detected in the serum after a short while. But despite the fast absorption, the total amount of urea absorbed was very small (Wohlrab & Schiemann, 1976:23). These findings confirm the belief that the stratum corneum forms a very effective barrier against the penetration of urea.

In another study by Wohlrab (1979:441) it was indicated that the permeation of 5-fluorouracil is accelerated by an admixture of, or pretreatment with urea (10 per cent w/v) and by increasing the hydration of the horny layer by pretreatment with 10 per cent w/v sodium chloride. The clinical effectiveness of 5-fluorouracil on epidermal disorders was increased simultaneously.

After biometrical, autoradiographical and cytophotometrical studies, Wohlrab (1976:589) noted upon five days application of urea that the epidermis was measurably thinned. It was concluded that the thinning of the epidermis after application of a saturated solution of urea in normal saline to the skin of a hairless white minipig could be attributed to the following processes: (1) the reduction of the number of cells being in DNA synthesis; (2) alteration of the generation of the post-mitotic epidermal cell; and (3) disturbances in the regulative mechanism while entering into the DNA synthesis or during its course.

3.3 THIOUREA

3.3.1 DESCRIPTION AND STABILITY

A synonym is thiocarbamide. The molecular structure of thiourea is $NH_2.CS.NH_2$ and the molecular weight is 76,12. It is a white crystalline solid with a melting point of 180°C and it behaves as a "monoacidic" base. It is stable in aqueous solution but will hydrolyse when heated with alkalis (Finar, 1967:422).

Thiourea is used to protect furs and clothing against insects.

3.3.2 DIFFUSION THROUGH MEMBRANES

Thiourea has often been used in diffusion studies to take part in structure relationship studies on the family of ureas. However, the results of Siegel (1981:139) indicate that the sulphur-containing thiourea penetrates the oral mucosa less readily than urea, an oxygen-containing compound. This deviation from Overton's rules has previously been noted by Diamond and Wright (1969:273) in the gall bladder, Naccache and Sha'afi (1973:714) in human red blood cells, Leaf and Hays (1962:921) in the urinary bladder and Treherne (1956:175) in the percutaneous absorption of thiourea through rabbit skin. A proper explanation for this phenomenon has not been encountered in this literature search.

3.4 GLYCEROL

3.4.1 DESCRIPTION AND STABILITY

Synonyms are glycerolum, glycerin and propane-1,2,3-triol. The molecular structure is $C_3H_8O_3$ and the molecular weight is 92,09. It is a clear, colourless, odourless, hygroscopic, syrupy liquid with a sweet taste followed by a sensation of warmth. Weight per millilitre is 1,255 to 1,26g, corresponding to 98 to 100 per cent of $C_3H_8O_3$.

It is miscible with water, alcohol and propylene glycol; slightly soluble in acetone; practically insoluble in chloroform, ether and fixed and volatile oils. It is incompatible with oxidizing agents.

A 10 per cent w/v solution in water is neutral to lithmus.
A 2,6 per cent w/v solution is iso-osmotic with serum (Martindale, 1977:626).

3.4.2 DIFFUSION THROUGH MEMBRANES

Siegel (1981:139) has found that glycerol with the three hydroxyl groups has a lower oil partition coefficient and permeability coefficient for oral mucosa than either 1,2-propanediol or 1,3-

propanediol. This may be related to glycerol's increased ability to form hydrogen bonds with water because of the third hydroxyl group. In a comparison of glycerol permeability and lipid composition of red blood cell membranes from eight mammalian species, Wessels and Veerkamp (1973:190) could find no correlation between lipid composition, including phospholipid and fatty acid composition, and the glycerol permeability coefficient.

Serban et al. (1981:429) have found that a 10 per cent v/v solution of glycerol produced hydration of the skin during the first few hours after application, mainly by diminishing the loss of water from the formulation. This is in agreement with the view of Murphy (1978:31) that under "moderate or high conditions of humidity, glycerin introduces moisture from the atmosphere to the skin".

It appears that glycerol has several properties which may limit its usefulness as a moisturizer. Under conditions of low relative humidity (ca 20 per cent) glycerol tends to lose water (Deshpande et al., 1980:20). Glycerol acts as a vehicle for moisture loss under conditions of low humidity by increasing the transepidermal water loss (Rieger & Deem, 1974:253). It also appears to be weakly held within the skin and is easily washed away (Murphy, 1978:31).

3.5 GLUCOSE

3.5.1 DESCRIPTION AND STABILITY

Synonyms are anhydrous dextrose, anhydrous glucose, purified glucose, grape sugar, glycosum, saccharum amylaceum and D-(+)-gluco-pyranose. The molecular formula is $C_6H_{12}O_6$ and the molecular weight is 180,2. It is a white odourless crystalline or granular powder with a sweet taste.

The solubility of glucose is approximately 1 in 1 of water and 1 in 200 of ethanol; it is soluble in glycerol and almost insoluble in ether.

A 5,05 per cent w/v solution in water is iso-osmotic with serum. Glucose is stable in an aqueous solution. It is a strong reducing agent (Finar, 1967:472).

3.5.2 Diffusion through membranes

The permeability of glucose through a variety of non-skin membranes has been studied (Stein, 1967:57). The penetration of glucose into the human epidermis was studied by Halprin and Ohkawara (1967:561). They came to the conclusion that the intercellular space of the epidermis comprises approximately 15 to 18 per cent of the total epidermal volume. Glucose not only fills this intercellular space but by a process of passive diffusion gains access to an additional 15 to 35 per cent of the intracellular volume of the epidermis in a concentration equal to the

blood glucose concentration or to all of the intracellular space at a concentration which is only 15 to 35 per cent of the blood glucose concentration. Their view that the extracellular fluid in the epidermis is "essentially water" is in conflict with the current view that intercellular spaces in the stratum corneum and epidermis contain large fractions of lipids (Elias & Friend, 1975:180). Whether or not the proposed lamellae character of the intercellular lipids, which maims watery layers, ties these two disparate ideas together is yet to be established.

The permeability coefficient of glucose was found to be extremely low for rabbit skin because of its very low lipid solubility as reflected in a very low ether-water partition coefficient (Treherne, 1956:175).

3.6 CONCLUSIONS

Because of the many positive correlations found between percutaneous absorption and oil-water partition coefficients (where "oil" is a general term for a hydrophobic solvent like ether, octanol etc.), it appears that many compounds penetrate the stratum corneum by dissolving in the lipids of the cell membrane and lipids between the cells.

An exception to this general behaviour is the deviation displayed by small hydrophilic molecules (Siegel, 1981:138). Wright and Pietras (1974:293) presented three explanations for the relatively fast permeation of small molecules. These include (1) permeation occurs through small "pores"; (2) permeation mediated by membrane

carriers; and (3) high permeability of small polar solutes as a consequence of the highly ordered configuration of plasma membrane lipids. Permeability coefficients remained constant over a wide range of concentrations of solute, which makes the second possibility unlikely. No direct evidence is available to distinguish between the first and third possibilities. The existence of streaming potentials across oral mucosa indicates the existence of polar groups in the epithelial membranes (Siegel et al., 1976:129). From the point of view of energetics, concentration of these polar groups in localized areas would yield the most stable membrane configuration (Wright & Diamond, 1969:227). The small polar molecules would thus prefer to interact with the polar groups in the membranes when diffusing through the stratum corneum. This view supports the mechanism that has been proposed by Scheuplein (1965:334).

The selected compounds in this study, (urea, thiourea, glycerol and glucose) are all small polar non-electrolytes but their physico-chemical properties differ enough with regard to hydrophilicity in order that a study of the relationship between percutaneous absorption and ether-water partition coefficients may be justified.

CHAPTER 4 THE RELATIONSHIP BETWEEN THE ETHER-WATER PARTITION COEFFICIENTS AND THE PERMEABILITY COEFFICIENTS OF COMPOUNDS THAT VARY IN HYDROPHOBICITY

4.1 INTRODUCTION

As early as 1899 Meyer and Overton recognized the importance of partitioning in the process of absorption of drugs through membranes. Since then, many workers have reported that increased hydrophobicity of a compound correlates with increased membrane permeability and generally increased biological activity (Valette et al., 1965:241; Treherne, 1956:171; Stoughton et al., 1960:337; Wurster and Kramer, 1961:288; Katz and Shaikh, 1965:291).

When investigating the relationship between ether-water partitioning and percutaneous absorption, one should select a biphasic in vitro partitioning system that will closely simulate the biphasic character of the membrane through which the permeation occurs. Water is the obvious choice for the polar phase and based on knowledge of biological membranes and lipids, a semi-polar solvent like diethyl ether should be a reasonable choice as second phase (Flynn, 1971:345).

The assessment of permeability coefficients by in vitro methods with sectioned or full thickness hairless mouse skin is well-established and a convenient way of studying the basic principles underlying percutaneous absorption. It was also the method used by other workers (Dürrheim et al., 1980:781; Smith, 1982:102), whose data will be used in the correlation of ether-water par-

tition coefficients and permeability coefficients of different series of compounds in this study.

The possitive correlations of the permeability of the straight chain alcohols and of other compounds with ether-water partition coefficients (Treherne, 1956:171; Stoughton et al., 1960: 337; Cronin and Stoughton, 1963:83) might have been fortuitous. Treherne (1956:171) for example, found a very poor quantitative agreement between the permeability coefficients and the ether-water partition coefficients. The permeability coefficients varied by about a factor ten while the partition coefficients varied by about a factor thousand.

The objective of this investigation by correlating ether-water partition coefficients with permeability coefficients is to determine the capacity of the skin to discriminate between the permeation of different compounds ranging in hydrophobicity. If the skin acts as a simple lipoidal barrier, the permeation will be a linear function of the hydrophobicity of the permeating substance. In this case the hydrophobicity is regarded as being reflected in the ether-water partition coefficient.

The permeability coefficients of the following compounds, as determined in vitro through hairless mouse skin, will be correlated with their ether-water partition coefficients: Urea, thiourea, glycerol, glucose, a homologous series of n-alkanols (methanol to n-octanol), hydrocortisone and a series of its 21-n-alkyl esters.

Data on the permeability coefficients and/or ether-water partition coefficients of these compounds were reported in the literature as indicated in table 4-1.

Table 4-1 Literature references on the permeability coefficients and/or ether-water partition coefficients of the compounds to be studied

Compound	Permeability coefficient in vitro through hairless mouse skin	Ether-water partition coefficient
Urea	-	Collander (1949:717) Ross (1951:229) Sandell (1966:330)
Thiourea	-	Collander (1949:717) Ross (1951:229) Sandell (1966:330)
Glycerol	-	Collander (1949:717) Ross (1951:229)
Glucose	-	Ross (1951:229)
Methanol to hexanol	Dürrheim et al. (1980:783)	Hansch & Leo (1979:169)
Heptanol and octanol	Dürrheim et al. (1980:783)	(1)
Hydrocortisone	Smith (1982:127)	Katz & Shaikh (1965:591) Nathansohn (1968:365) Flynn (1971:349)
Hydrocortisone 21-acetate	Smith (1982:127)	Katz & Shaikh (1965:591) Mantica et al. (1970:109) Flynn (1971:349)
Hydrocortisone 21-propionate	Smith (1982:127)	-
Hydrocortisone 21-butyrate	Smith (1982:127)	Flynn (1971:349)
Hydrocortisone 21-pentanoate	Smith (1982:127)	-
Hydrocortisone 21-hexanoate	Smith (1982:127)	Flynn (1971:349)
Hydrocortisone 21-heptanoate	Smith (1982:127)	-

(1) Could be extrapolated from data on methanol to hexanol (Smith, 1982)

In order to obtain a complete set of data on both the permeability coefficients and the ether-water partition coefficients of the abovementioned compounds, it was necessary to assess the permeability coefficients of urea, thiourea, glycerol and glucose and the ether-water partition coefficients of some of the hydrocortisone 21-n-alkyl esters.

The results of the investigations into the permeability of urea, thiourea, glycerol and glucose through hairless mouse skin *in vitro* are presented under paragraph 4.3. Although all the partition coefficients which are necessary for the correlation of permeability and ether-water partitioning, are available in the literature or can be calculated from the available literature, the ether-water partition coefficients of urea, thiourea, glycerol and glucose and hydrocortisone and its 21-n-alkyl esters were determined in this study. The reasons why this was done, are given under paragraph 4.2.

4.2 DETERMINATION OF ETHER-WATER PARTITION COEFFICIENTS

The ether-water partition coefficients of the selected hydrophilic compounds, urea, thiourea, glycerol and glucose have been determined by several workers (Collander, 1949:717; Ross, 1951: 229; Sandell, 1966:330). They determined these values by means of rather outmoded laborious techniques. It was therefore decided to assess the utility of a very simple radiotracer technique in this regard while simultaneously assessing the validity of the existing ether-water partition coefficients of some compounds critical to the analysis.

It was also decided to determine the ether-water partition coefficients of hydrocortisone and its 21-n-alkyl esters (up to hydrocortisone-21-heptanoate) by means of an HPLC method (Smith, 1982:112). This was done to supplement existing data and as a check on the results of Flynn (1971:349), Katz and Shaikh (1965: 591) and Mantica et al. (1970:109).

4.2.1 LOW MOLECULAR WEIGHT, HYDROPHILIC COMPOUNDS (UREA, THIOUREA, GLYCEROL AND GLUCOSE)

The ether-water partition coefficients of urea and thiourea were reported by all three of the above-mentioned authors, but those of glycerol only by Collander (1949:717) and Ross (1951:229) and those of glucose were only approximated by Ross (1951:229). The radiotracer method should be sensitive enough to determine the partition coefficient of glucose more accurately and reliably enough to supplement and validate the existing data.

4.2.1.1 EXPERIMENTAL

4.2.1.1.1 MATERIALS

D-(1-^{14}C) Glucose[1], supplied in a sterile aqueous solution containing 3 per cent alcohol.

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[1]Amersham Corporation, Arlington Heights, Illinois.

(1,3-^{14}C)-Glycerol[1], supplied as a sterile aqueous solution.
(^{14}C)-Thiourea[2], supplied as a powder and made up in reagent grade ethanol.
(^{14}C)-Urea[2], supplied as an ethanol solution.

Double-distilled water and analytical reagent grade ether[3] were used to prepare the co-saturated phases. See table 4-2 for more information on the radio-labelled compounds.

4.2.1.1.2 PROCEDURE

Water and ether were added to a 120 ml separator and shaken to obtain co-saturation of the phases. The separator was set aside for 24 hours to allow for temperature and further phase equilibrium. All remaining steps were carried out at room temperature (23±1°C). Two millilitres of the water phase were placed in a scintillation vial[4] by means of a micropipette[5]. The weight of the vial plus the 2 ml water phase was recorded. An appropriate amount of the ether phase was placed in the vial (2-4 ml). A sample of the radio-labelled material was placed in the vial (20-50 ul) my means of a micropipette. The vial was thoroughly shaken by hand for 2

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[2] New England Nuclear, Boston, Massachusetts.

[3] MCB Manufacturing Chemists, Inc., Cincinnati, Ohio.

[4] Disposable Kimble Vials (20 ml Borosilicate Glass).

[5] Finnpipette, Variable modules, Labsystems OY, Helsinki, Finland.

Table 4-2 The stuctural formula, formula weight, specific activity and radioactive concentration of the radiolabeled glucose, glycerol, thiourea and urea used in the partitioning and permeation studies.

Compound	Structural formula	Formula weight	Specific activity mCi/mmol	Radioactive concentration μCi/ml
D-(1-^{14}C)Glucose	$\overset{*}{C}HO(CHOH)_4CH_2OH$	182,0	61,1	200
(1,3-^{14}C)-Glycerol	$HO\overset{*}{C}H_2CHOH\overset{*}{C}H_2OH$	92,1	43,4	100
(^{14}C)-Thiourea	$H_2N\overset{*}{C}SNH_2$	76,1	47,5	400
(^{14}C)-Urea	$H_2N\overset{*}{C}ONH_2$	60,1	53,5	500

* Radio-active labeled carbon atoms.

minutes and allowed to stand for 20-30 minutes to assure complete phase separation.

Because of the volatility of the ether, the weight of the vial plus contents was recorded just before the samples were taken in order to calculate the volume of the ether phase. The samples from the ether and water phases were taken with great care not to disturb the phase equilibrium. Three samples per phase per vial were taken. The samples were put in 10 ml scintillation coctail[6] and analysed with a scintillation counter[7].

4.2.1.2 RESULTS

The ether-water partition coefficients of the selected hydrophilic compounds as determined by means of a simple radiotracer procedure are listed in table 4-3.

4.2.1.3 DISCUSSION AND CONCLUSIONS

The log of the ether-water partition coefficients obtained in this study is listed together with those of other workers in table 4-4.

When the ether-water partition coefficients obtained in this study are compared to those of other workers, there seems to be an excellent correlation between the values of thiourea and urea and a

[6] Aquasol, New England Nuclear, Boston, Massachusetts.

[7] Beckman LS 9000 Liquid Scintillation Systems, Beckman Instruments, Ins., Fullerton, California.

Table 4-3 The ether-water partition coefficients of glucose, urea, glycerol and thiourea as determined by a radiotracer procedure

Compound	Partition coefficient	Standard deviation [1]	Log partition coefficient
Glucose	$8,7 \times 10^{-6}$	$2,7 \times 10^{-6}$ (5)	-5,06
Urea	$3,2 \times 10^{-4}$	$0,7 \times 10^{-4}$ (6)	-3,49
Glycerol	$5,1 \times 10^{-4}$	$0,9 \times 10^{-4}$ (6)	-3,29
Thiourea	$7,2 \times 10^{-3}$	$2,3 \times 10^{-3}$ (5)	-2,14

[1] Number of determinations in brackets.

Table 4-4 The log of the ether-water partition coefficients obtained in this study for glucose, urea, glycerol and thiourea and those by other authors.

Compound	Log partition coefficient	Reference
Glucose	-5,06	This study
	< -4,00	Ross (1951:229)
Urea	-3,49	This study
	-3,52	Sandell (1966:330)
	-3,30	Ross (1951:229)
	-3,33	Collander (1949:717)
Glycerol	-3,29	This study
	-2,96	Ross (1951:229)
	-3,18	Collander (1949:717)
Thiourea	-2,14	This study
	-2,10	Sandell (1966:330)
	-2,14	Ross (1951:229)
	-2,20	Collander (1949:717)

fair correlation in the case of glycerol. The ether-water partition coefficient of glucose was only reported by Ross (1951) who gave only a maximum value because his analytical methods were not sensitive enough to determine the extremely low concentration of glucose in the ether phase.

It can be concluded that the simple radiotracer technique that was used in this study gave reproducible results which compared very well to values found by earlier workers who used other techniques. In the case of glucose, the radiotracer technique seems to be sensitive enough for an accurate estimation of the ether-water partition coefficient and it seems to be of superior sensitivity to the methods used by previous authors.

The radiotracer method is not only very sensitive to very low concentrations but also very simple and economical both as to time and reagents.

4.2.2 HYDROCORTISONE AND A SERIES OF HYDROCORTISONE 21-n-ALKYL ESTERS

The ether-water partition coefficients of hydrocortisone were reported by Katz & Shaikh (1965:591) Nathansohn (1968:365) and Flynn (1971:349), those of hydrocortisone-21-acetate by Katz & Shaikh (1965:591), Mantica et al. (1970:109) and Flynn (1971:349) and those of hydrocortisone-21-butyrate and hydrocortisone-21-hexanoate only by Flynn (1971:349).

The values found by the different workers differed substantially

and except for hydrocortisone and hydrocortisone-21-acetate the data on the ether-water partition coefficients of the other hydrocortisone 21-n-alkyl esters were only assessed by Flynn (1971: 349) and were otherwise unavailable in the literature (see table 4-6).

The objective of the ether-water partition coefficient determinations was to supplement the information available in the literature and to verify the values found by previous workers. This was because this series of compounds can be considered prototypical of the corticosteroids and therefore the permeability of all the compounds through mouse skin was actively researched by Smith (1982:100). The ether-water partition coefficients of the whole series of hydrocortisone and its 21-n-alkyl esters were to be determined by means of a HPLC method. This method obviated concerns of impurity of the compounds as it is molecularly specific.

4.2.2.1 EXPERIMENTAL

4.2.2.1.1 MATERIALS

Hydrocortisone was obtained from the Upjohn Company[8] and was used without further purification. The hydrocortisone 21-n-alkyl esters (acetate through to heptanoate) shown in figure 4.1 were synthesized as outlined by Hagen (1979:119) and Smith (1982:102) by reacting an excess of the anhydride of a specific acid with

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[8] Upjohn Company, Kalamazoo, Michigan.

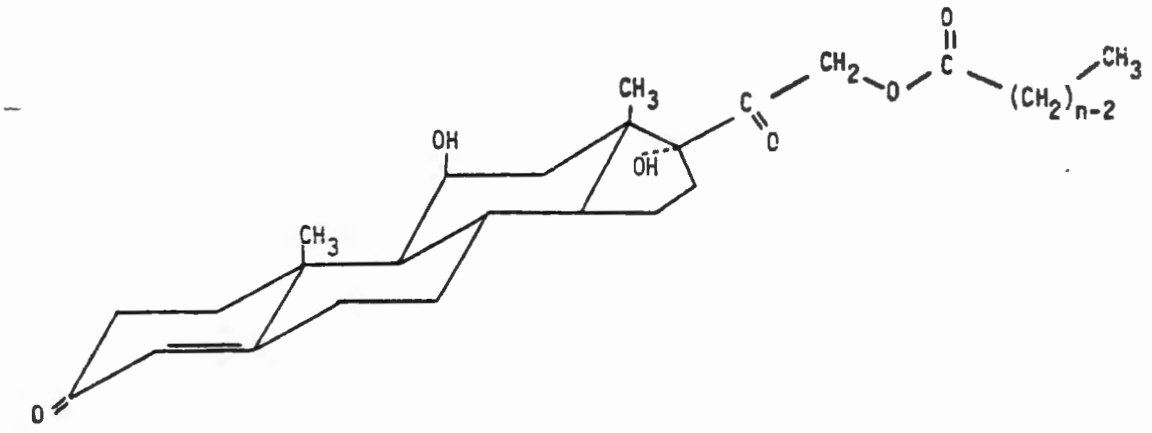

Figure 4-1 Structural configuration of the homologous hydrocortisone 21-n-alkyl esters (Hagen, 1979:121).

5 g of hydrocortisone in 100 ml of pyridine.

Double-distilled water and analytical reagent ether[9] were used to prepare the co-saturated phases.

4.2.2.1.2 PROCEDURE

Water and ether were added to a 2 litre separator and shaken to obtain co-saturation of the phases. The separator was set aside for 24 hours to allow for temperature and further phase equilibrium. All remaining steps were carried out at room temperature ($23\pm1^{0}C$). An appropriate amount of steroid (from 1-10 mg, depending on the steroid) was accurately weighed and put into a 50 ml conical flask. This was dissolved in the ether phase, which was maintained at approximately 25 to 50 ml. An appropriate amount of water phase (25-500 ml depending on the solubility of steroid) was placed in an appropriately sized separator (60-1000 ml). The steroid solution in the ether phase was added with no attempt to rinse any residual steroid from the flask or funnel used. The phases were shaken intermittently, allowing sufficient time between agitations for the phases to reform.

The system was allowed to stand for 20 to 30 minutes to assure complete phase separation. Then the aqueous phase was transferred to a clean, appropriately-sized separator with great care taken to assure that no ether phase contaminated it at this point. The ether phase was immediateli transferred to a 100 ml round bottom flask, the phase weight was recorded and the phase was

[9] MCB Manufacturing Chemists, Associate of E. Merck, Darmstadt, Germany, 2909 Highland Ave., Cincinnati, Ohio.

evaporated to dryness on a Buchi evaporator[10]. The aqueous phase was extracted with four 25 ml portions of methylene chloride and the extracts were collected in a 250 ml round bottom flask. The combined extracts were then evaporated to dryness on the evaporator. The dried-phase residues were reconstituted with reagent grade methanol[11] (usually 5-25 ml of methanol for the aqueous phase residue and 25-50 ml of methanol for the ether phase residue) and assayed by the following HPLC procedure (Hagen. 1979:121).

A Waters Assosiates solvent delivery system[12] and absorbance detector[13] were used with a reverse phase high efficiency liquid chromatography column[14]. The mobile phase was a 70:30 mixture of reagent grade methanol and double-distilled water. The flow rate of the mobile phase was 1,0 to 1,5 ml/min. The attenuation reference used was 0,1, the injection volume was 50 µl and the chart speed 0,25 cm/min. An Omniscribe recorder[15] was used. The peak heights of the samples were compared to a concentration standard curve. The separation of hydrocortisone and its 21-n-alkyl esters on an HPLC chromatogram is shown in figure 4-2.

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[10] Buchi Rotavapor-R, Brinkman Instruments, Westbury, New York.

[11] Methanol Anhydrous Reagent ACS, MCB Manufacturing Chemists, Cincinnati, Ohio.

[12] Model 6000A, Waters Assosiates, Inc., Massachusetts.

[13] Model 440, Waters Assosiates Inc.

[14] µBondapak C_{18}, Waters Assosiates Inc.

[15] Omniscribe $_{TM}$ Series B-5000, Houston Instrument Texas.

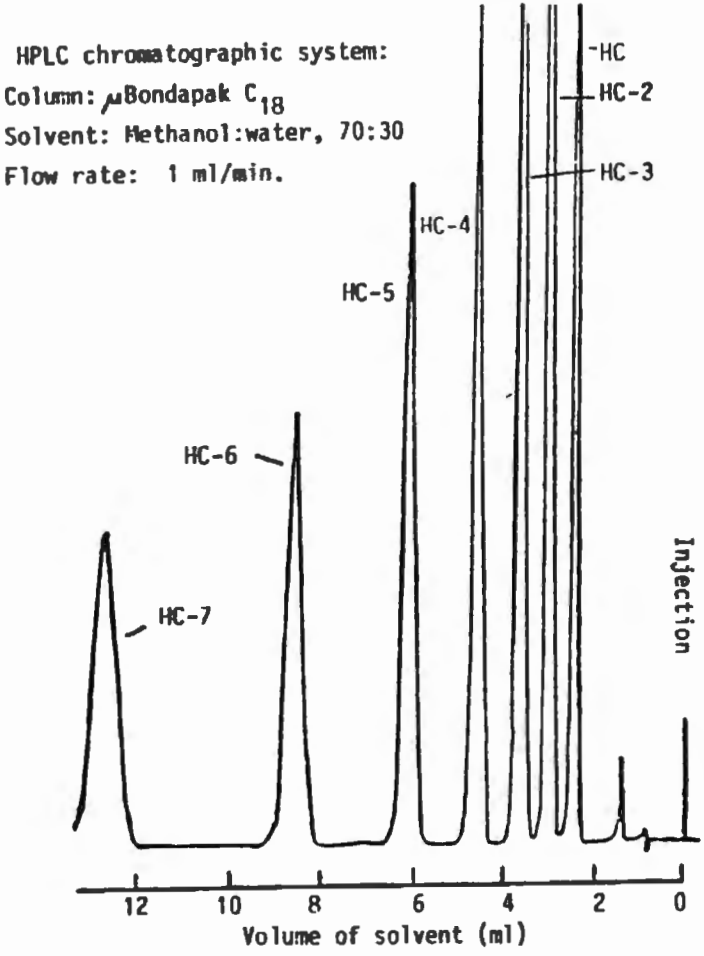

Figure 4-2 Seperation of hydrocortisone and its 21-n-alkyl esters on an HPLC chromatogram under the specified conditions (unequal concentrations).

4.2.2.2 RESULTS

The ether-water partition coefficients of hydrocortisone and the series of hydrocortisone 21-n-alkyl esters is listed in table 4-5.

4.2.2.3 DISCUSSION AND CONCLUSIONS

The log of the obtained ether-water partition coefficients is listed together with those of other workers in table 4-6.

All the ether-water partition coefficients obtained in this study are in general of the same order as those obtained by others. In the case of hydrocortisone the value obtained in this study is nearest to that obtained by Nathansohn et al. (1968:365). The value found for hydrocortisone acetate is almost identical to that obtained by Flynn (1971:349). The values obtained in this study for hydrocortisone-21-butyrate and hydrocortisone-21-hexanoate are a little higher than those obtained by Flynn (1971:349).

The parameter π as defined by Fujita et al. (1964:5175), which describes the partitioning tendencies of substituents, can be used to calculate the π_{CH_2} values for the series of hydrocortisone 21-n-alkyl esters.

When the ether-water partition coefficients determined for hydrocortisone and its 21-n-alkyl esters (table 4-5) are plotted semi-logarithmically as a function of alkyl chainlength a straight line relationship is found and the slope of the plot is the π value (figure 4-3).

Table 4-5 The ether-water partition coefficients of hydrocortisone and its 21-n-alkyl esters.

Compound	Partition coefficient	Standard deviation [1]	Log partition coefficient
Hydrocortisone (H)	1,42	0,08 (8)	0,15
H-21-acetate	26,9	0,6 (4)	1,43
H-21-propionate	94,7	2,5 (3)	1,98
H-21-butyrate	303	19 (3)	2,48
H-21-pentanoate	905	61 (3)	2,96
H-21-hexanoate	4048	85 (3)	3,61
H-21-heptanoate	13707	3411 (4)	4,14

[1] Number of determinations in brackets.

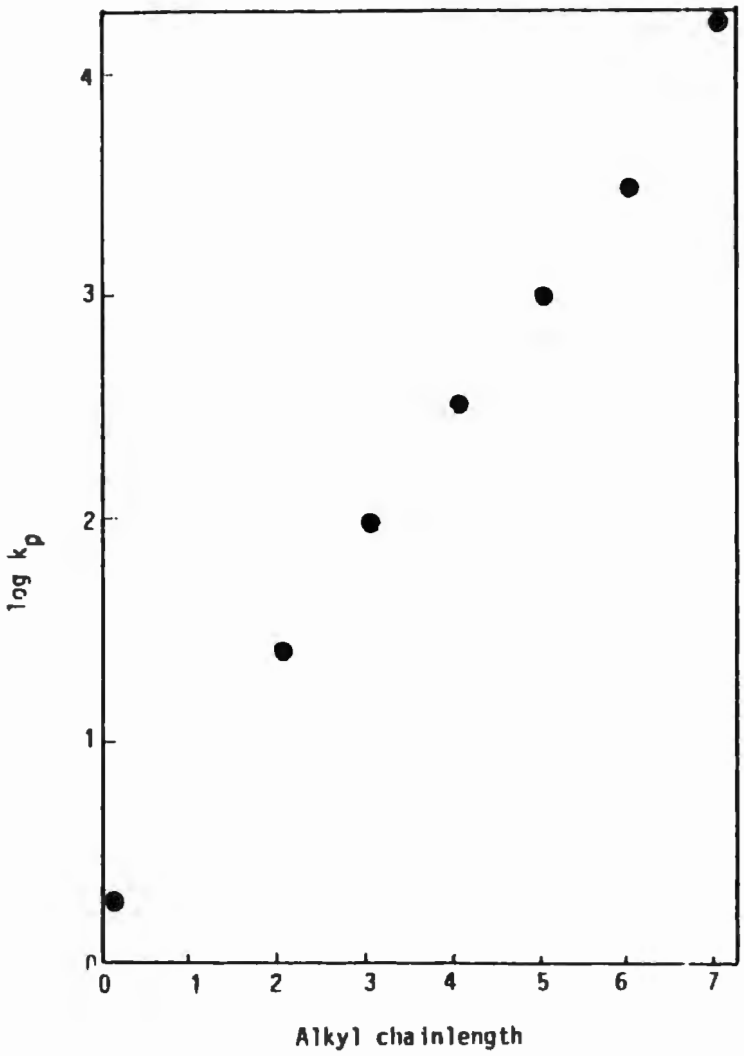

Figure 4-3 The logarithm of the ether-water partition coefficient (k_p) as a function of alkyl chainlength for hydrocortisone and its 21-n-alkyl esters.

Table 4-6 The log of the ether-water partition coefficients (log k_p) of hydrocortisone and its 21-n-alkyl esters obtained in this study and those of other authors

Compound	Log k_p	Reference
Hydrocortisone	0,15	This study
	0,21	Flynn (1971:349)
	0,18	Nathansohn et al. (1968:365)
	0,11	Katz and Shaikh (1965:591)
Hydrocortisone-21-acetate	1,43	This study
	1,42	Flynn (1971:349)
	1,09	Mantica et al. (1970:109)
	1,11	Katz and Shaikh (1965:591)
Hydrocortisone -21-propionate	1,98	This study
Hydrocortisone-21-butyrate	2,48	This study
	2,37	Flynn (1971:349)
Hydrocortisone-21-pentanoate	2,96	This study
Hydrocortisone-21-hexanoate	3,61	This study
	3,56	Flynn (1971:349)
Hydrocortisone-21-heptanoate	4,14	This study

The π values found in this study and according to the data of Flynn (1971:349) are 0,542 and 0,536 with correlation coefficients of 0,9992 and 0,9985 respectively. The excellent correlation found in this study is indicative of the reliability of the HPLC method. Consequently it can be concluded that the ether-water partition coefficients of hydrocortisone and its derivatives found in the literature were relatively reliable estimates easily and accurately verified in this work.

4.3 ASSESSMENT OF THE PERMEABILITY COEFFICIENTS OF UREA, THIOUREA, GLYCEROL AND GLUCOSE THROUGH HAIRLESS MOUSE SKIN *IN VITRO*

4.3.1 INTRODUCTION

As indicated in table 4-1, no permeability coefficients have been determined for the abovementioned hydrophilic compounds with regard to the *in vitro* permeation of hairless mouse skin. A previous worker (Treherne, 1956:171) used rabbit skin, a highly questionable membrane choice. In order to make possible an inquiry into the relationship between the permeability coefficients and ether-water partition coefficients of these compounds, it was necessary to obtain accurate permeability coefficients in a more acceptable model system like the hairless mouse system.

Because these data will be considered together with those on a series of alkanols and on hydrocortisone and its 21-n-alkyl esters, all obtained using the same basic *in vitro* procedure and

hairless mouse skin as developed by DÜrrheim et al. (1980:781) and employed by Smith (1982:100).

4.3.2 EXPERIMENTAL

All the permeation studies were done by determining the permeation profiles of the radio-labelled hydrophilic compounds through full thickness or sections of hairless mouse skin in a diffusion cell system. Sink conditions were maintained throughout all determinations.

4.3.2.1 MATERIALS

The same stock solutions of radio-labelled compounds as described under 4.2.1.1.1 were used. The concentrations in which the different compounds were used are listed in table 4-7. The analytical grade of unlabelled urea[16], glycerol[17] and glucose[17] was also used. All the abovementioned compounds were used as received with no attempt to confirm or increase the purity. All stock solutions were kept in a refrigerator. Stock solutions of the unlabelled compounds were freshly made up before each experiment. The diffusional media used in these experiments were prepared from 0,9 per cent sodium chloride for irrigation, henceforth referred to as normal saline[18] because of its isotonic

[16] Mollincrodt, St. Louis, Missouri.

[17] Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, New Jersey.

[18] Normal Saline for Irrigation, Abbott Laboratories, North Chicago, Illinois.

Table 4-7 Concentrations in which glycerol, urea, glucose and thiourea were used in the permeation studies

Compound	Concentration unlabelled compound in donor and receiver cells	Concentration labelled compound in donor responsible for concentration gradient
Glucose	0,01 M	$3,33 \times 10^{-6}$ M
Urea	0,01 M	$9,16 \times 10^{-6}$ M
Glycerol	0,01 M	$5,75 \times 10^{-6}$ M
Thiourea	-	$2,63 \times 10^{-5}$ M

character and also because of its extensive use in permeation experiments on the alkanols and hydrocortisone and its 21-n-alkyl esters.

4.3.2.2 MOUSE SKIN MEMBRANE PREPARATIONS

The abdominal skin from the hairless mouse[19] was used exclusively in this study. Male mice over the age of 90 days were used since studies by Behl and co-workers have showed that the age-related variability of alkanol permeability coefficients could be minimized by using older animals (Smith, 1982:103).

The mice were killed by severing the spinal cord. Four abdominal sections of full thickness skin, each about 1,5 square centimeter in area were removed from the mouse by blunt dissection (figure 4-4) and mounted between the cells of four glass diffusion cell systems (figure 4-5). The necessary precautions were observed during the preparation and execution of each experiment that the skin was not been subjected to stretching and too much stress during manipulations like rinsing and filling of the diffusion cells.

Dermal tissue was obtained in the following manner: Full thickness abdominal hairless mouse skin, mounted in a large diffusion cell system (4,9 cm^2 exposed to diffusion medium and 20 ml capacity) was soaked in normal saline at 37^0C for 12 hours. The skin was removed and the epidermis peeled off, using two micropipette tips as tweezers. The dermis was then mounted in a small

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[19] SKH-hr-1, Skin Cancer Hospital, Temple University, Philadelphia, Penssylvania.

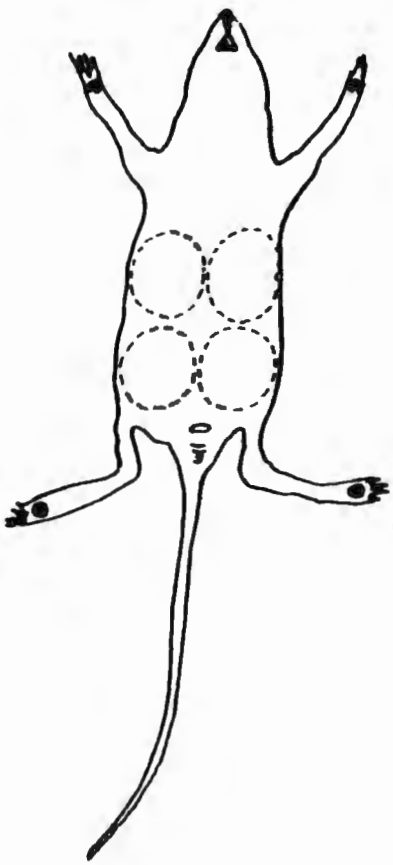

Figure 4-4 Schematic diagram of the sections of hairless mouse abdominal skin used for all the experiments with the small diffusion cells. Diameter of the diffusional area is approximately one centimeter.

diffusion cell system (0,7128 cm^2 exposed to diffusion medium and 1,3 ml volume). Immediately after this procedure the dermis experiment was started.

4.3.2.3 GENERAL PROCEDURES

The diffusion cell system consisted of two cells with a volume of approximately 1,3 ml each. When the skin is mounted (clamped) between the cells an area of 0,7128 cm^2 of skin is exposed to the contents of the cells. The contents of each cell were stirred by a small propeller-type stirrer.

The diffusion cell system was mounted in a waterbath in which the temperature was maintained at $37 \pm 1^{o}C$. The stirrers were connected to motors[20] which maintained the stirring speed at 150 rotations per minute. All the experimental variables e.g. temperature and stirring speed, were checked at regular intervals during each experiment.

The cell in which the high concentration of compound was put, was called the donor cell and the other cell in which the concentration was kept as low as possible was called the receiver cell. The essentials of a sink condition were maintained throughout

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[20] Model CA115V constant-speed motor (150 rpm), Hurst Manufacturing Co., Princeton, Indiana.

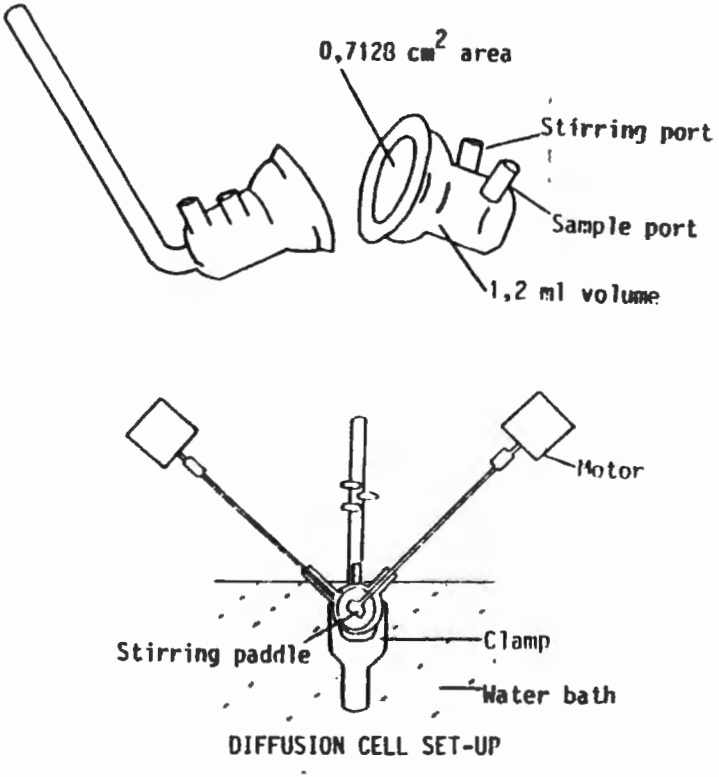

Figure 4-5 Glass diffusion cells and experimental set-up used for the permeation experiments.

each experiment, which means that the receiver cell should contain an infinitely small concentration of the diffusing compound in comparison to the concentration in the donor cell. The limit was taken that the receiver cell should not contain a higher concentration than five per cent of the donor cell concentration of radio-labelled compound. This means that the thermodynamic driving force was not allowed to drop below 90 per cent of the original level. After each piece of skin was excised and clamped between the two cells, the cell system was put into the waterbath and 1,2 ml of a normal saline solution (prewarmed to 37°C) was put into each cell.

After all four cells systems were put in the waterbath, the stirrers were connected to the motors. The skin was bathed in this manner for a half hour to one hour before the experiment commenced.

As necessary to exchange the medium in either cell, the contents of the cells were removed by means of a syringe with a piece of plastic tubing connected to the needle for easier evacuation of the cell. In all experiments a standard volume of 1,2 ml was put in each cell. Samples of 20 µl and 400 µl were respectively taken from the donor and receiver cells at specified time intervals and replenished accordingly. The samples were put in 10 ml of a scintillation coctail[21] before being analysed in a scintillation counter[22].

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[21] Aquasol, New England Nuclear, Boston, Massachussets.

[22] Model LS 9000 liquid scintillation counter, Beckman Instruments, Fullerton, California.

All the manipulations that required the measuring of accurate volumes, were done by means of micropipettes[23]. Between manipulations the sample-taking ports were closed by means of micropipette tips of which the tips were wrapped in Parafilm[24] before using them as stoppers for the ports.

4.3.2.4 CALCULATION OF THE PERMEABILITY COEFFICIENTS

The theory underlying the calculation of permeability coefficients has already been thoroughly discussed (Aquiar and Weiner, 1969: 210; Dürrheim, 1977:57; Flynn, 1979:289; Smith, 1982:48).

Since sink conditions were maintained throughout each experiment, the equation derived from Fick's law could be used for the calculation of the permeability coefficients. The permeability data were plotted as counts (amount of permeant) collected in the receptor compartment as a function of time. Correction was made for sampling, which was done by replacement. The permeability coefficient at different points in a run was calculated from:

$$J_T = PA\Delta C \qquad \text{(Eq. 1)}$$

where J_T is the total flux and also the slope of plots of the amount penetrated versus time (counts per minute); P is the permeability coefficient (centimeters per hour); A is the diffusional area (square centimeters); and ΔC is the concentration

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[23] Variable module Finnpipettes. Manufactured by Labsystems, Helsinki, Finland. Obtained from Markson Science, Chicago, Illinois.

[24] Parafilm, American Can Company, Greenwich, Cincinnati, Ohio.

differential across the membrane, which was taken to be equal to the donor phase concentration (counts per cubic centimeter). It should be noted that J_T is to be differentiated from J, the flux per unit area, as used earlier. The concentrations were expressed in terms of counts from a 400-µl sample. Thus, the permeability coefficient is calculated from:

$$P = \frac{J_T}{A\,\Delta C} \qquad \text{(Eq. 2)}$$

and since $J_T = dM/dt = V(dC/dt)$:

$$P = \frac{V\,\frac{dC}{dt}}{A\,\Delta C} \qquad \text{(Eq. 3)}$$

where V is the half-cell volume and the volume of the receptor compartment (cubic centimeters) and dC/dt is the steady-state slope in counts per minute per cubic centimeter. The computed permeability coefficients were converted to centimeters per hour by multiplying by 60.

4.3.3 RESULTS

The typical permeation profile found with thiourea is presented in figure 4-6. The insert on the graph represents the detailed profile over the first four hours. There is a definite biphasic character to the profile.

An example of the typical donor and receiver cell concentrations

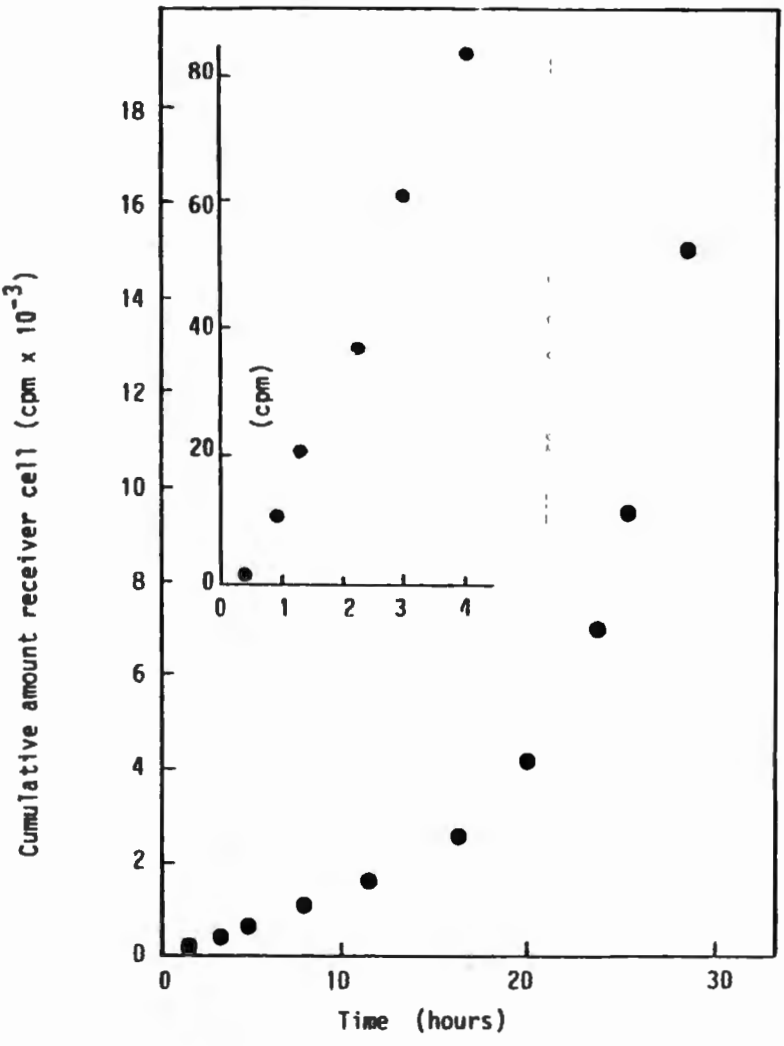

Figure 4-6 The cumulative amount of (^{14}C)-Thiourea (counts per minute) in the receiver cell as a function of time when permeated through full thickness hairless mouse skin.

for urea over a period of 30 hours is presented in table 4-8.

The permeability coefficients of these compounds when permeating full thickness and the dermis of hairless mouse skin are presented in table 4-9.

4.3.3 DISCUSSION AND CONCLUSIONS

With urea, glycerol and glucose an acceptable, reproducible profile could not be obtained by using only a tracer amount of radio-labelled material in the donor cell. Although a decrease of concentration in the donor cell could be measured, the concentration in the receiver cell did not reflect good mass balance. It was concluded that these compounds were being sorbed and bound or metabolised and bound in the skin or on the surfaces of the diffusion cell.

It was found that by putting a 0,01 M unlabelled solution of the compound in both the receiver and donor cells, the sorptive capacity of the cell system, including the skin, was saturated and the permeation of the radio-labelled compound could easily be followed. After addition of unlabelled solutions of urea, glycerol and glucose, essentially the same permeation profile as obtained with thiourea was observed for these compounds.

It seemed as if a mass balance was almost immediately obtained at the start of the experiment and that the typical lag time phenomenon (Dürrheim et al., 1980:783; Smith, 1982:125) was absent in the case of these compounds. Because of the very slow permeation rate of the hydrophilic compounds over the first few

Table 4-8 An example of the typical donor and receiver cell concentrations for urea over a period of 30 hours

Time (hours)	Concentration in donor cell (counts per minute)[1]	Concentration in receiver cell (counts per minute)[2]
10	712 950	1 043
20	711 400	3 383
30	733 500	6 156

[1] The calculated average concentration in a 400 µl sample used as the concentration differential (ΔC) in the calculation of the permeability coefficient.

[2] Without correction for dilution in a 400 µl sample.

Table 4-9 The permeability coefficients of glucose, urea, glycerol and thiourea for full thickness hairless mouse skin (first 6 hours) and dermis of hairless mouse skin

Compound	Permeability coefficient full thickness (cm/hr)	Permeability coefficient dermis (cm/hr)
Glucose	$9,5 \times 10^{-5}$	0,29
Urea	$1,2 \times 10^{-4}$	0,68
Glycerol	$1,4 \times 10^{-4}$	0,41
Thiourea	$9,6 \times 10^{-5}$	0,62

hours, an extemely slow build-up of radio-labelled compound occurs in the receiver cell. There is therefore a problem with the accuracy of experimental data over the first six to ten hours of an experiment due to the difficulty in detecting the exact concentration of the compound in the receiver cell.

Another way of determination of the permeation profile would have been to measure the loss from the donor cell instead of the gain in the receiver cell. But the concentration in the donor cell has to be much higher than that in the receiver cell to provide a sufficient concentration gradient over the skin and the disappearance of a small amount of radio-labelled compound (e.g. 100 counts per minute) from a very high amount (e.g. 400 000 counts per minute) is almost undetectable. A very small error in sample-taking from the donor, e.g. a 0,25% error would present a difference of 1000 counts per minute on 400 000 counts per minute. The magnitude of the abovementioned problem is increased by the variation in the counting of the scintillation counter.

An interesting phenomenon encountered with all four of the studied hydrophilic compounds, was a continuously increasing permeation rate as a function of time. The permeability coefficients as calculated over the first six hours and last six hours of a 30 hour run of each of the compounds are presented in figure 4-7 as a function of their ether-water partition coefficients. This was a phenomenon not encountered previously with water and the n-alkanols. For that matter, the same tissue was "stable" to the penetration of corticosteroids for well over a day's time.

A remarkable similarity is found when the initially estimated permeability coefficients obtained in this study are compared

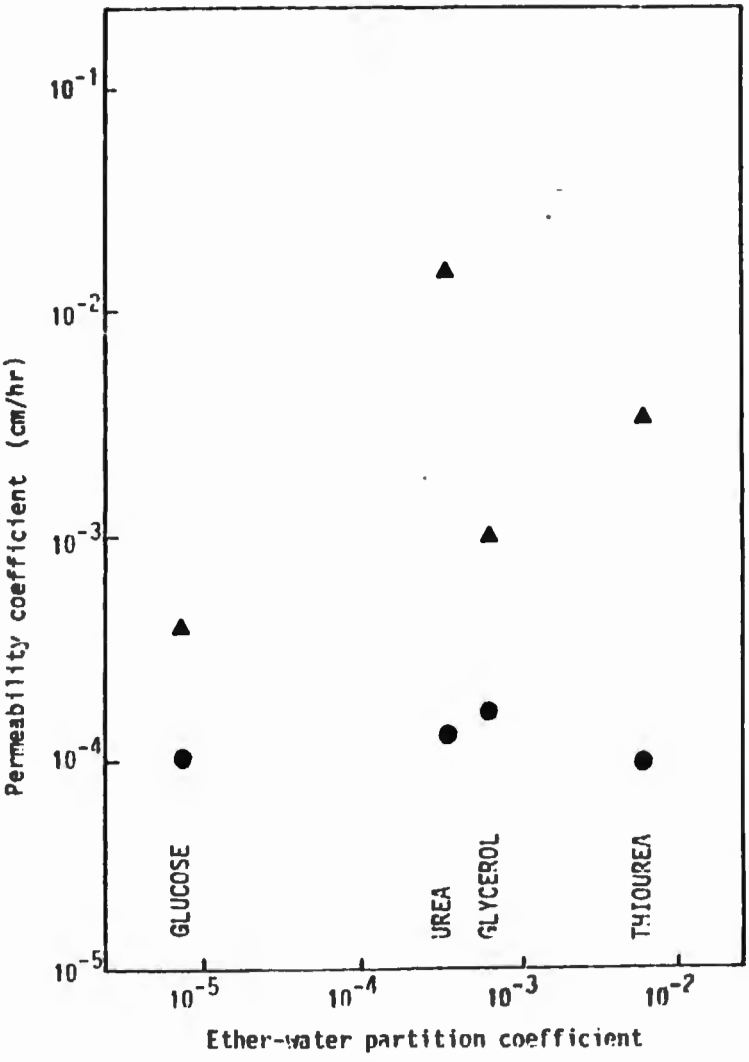

Figure 4-7 The permeability coefficients as calculated over the first six hours (●) and last six hours (▲) of a 30-hour run for glucose, urea, glycerol and thiourea as a function of the ether-water partition coefficients.

to those obtained by Treherne (1956:171). He used the same compounds but his membrane was excised rabbit skin. The initial estimates were those based on the first 6 hours of the experiments. See figure 4-8 for the values found in this study and those of Treherne (1956:171) for full thickness skin and dermis.

Although the quantitative values for rabbit dermis (Treherne, 1956:175) are approximately ten times smaller than those found for hairless mouse dermis, exactly the same tendency is found for both species when permeability is taken as a function of the ether-water partition coefficients of the hydrophilic compounds. There appears to be very little dependency of permeability on the polarity of the four compounds that were studied.

It can be concluded that when only the first six hours of the experiments are taken into account, the permeability coefficients of urea, thiourea, glycerol and glucose for full thickness hairless mouse skin are very low and closely grouped between 1×10^{-4} and $1,4 \times 10^{-4}$ cm/hr. The changing permeability of these compounds with time and especially past 6 hours is dealt with in a later chapter. The permeability coefficients found for the dermis are grouped between 0,3 and 0,7 cm/hr, which represent in general higher values than the highest value of 0,32 cm/hr found for methanol by Dürrheim (1977:134).

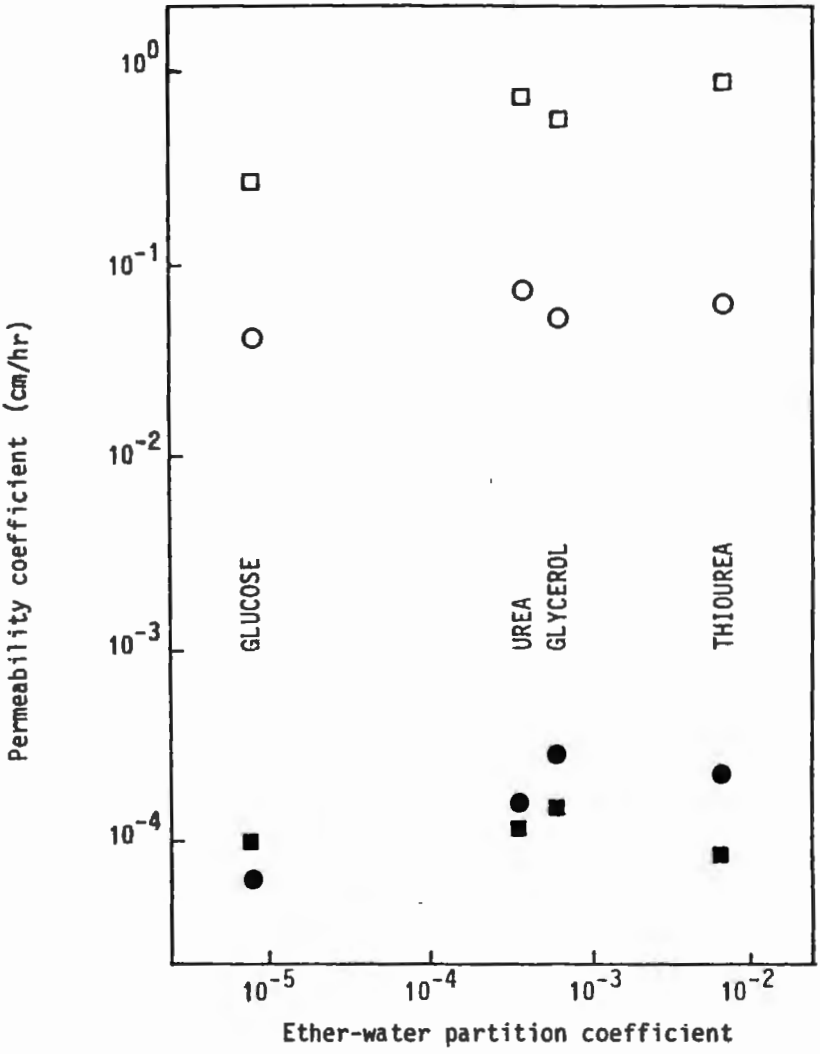

Figure 4-8 The permeability coefficients of glucose, urea, glycerol and thiourea for full thickness rabbit skin (●)(Treherne, 1956); full thickness hairless mouse skin (■)(This study); dermis of rabbit (○)(Treherne, 1956); dermis of hairless mouse (□)(This study) as a function of the ether-water partition coefficient.

4.4 DISCUSSION ON THE RELATIONSHIP BETWEEN THE ETHER-WATER PARTITION COEFFICIENTS AND PERCUTANEOUS ABSORPTION THROUGH HAIRLESS MOUSE SKIN OF COMPOUNDS RANGING MUCH IN HYDROPHOBICITY

The objective when comparing the permeability coefficients of compounds ranging so widely in hydrophobicity to their ether-water partition coefficients is to see whether a pattern emerges which can be used as a guide to the possible mechanisms by which these compounds traverse the skin and particularly the stratum corneum. It that could be done, the ether-water partition coefficient of a compound can be used as a parameter in estimating its permeability coefficient through the skin.

The ether-water partition coefficients of urea, thiourea, glycerol and glucose, a series of alkanols and hydrocortisone and its 21-n-alkyl esters and their permeability coefficients through hairless mouse skin *in vitro* are listed in table 4-10. These values are plotted on a log-log scale in figure 4-9.

The permeability coefficients of the hydrophilic compounds (glucose, urea, glycerol and thiourea) vary only from 1×10^{-4} to $1{,}4 \times 10^{-4}$ cm/hr while their ether-water partition coefficients vary from 9×10^{-6} to $7{,}2 \times 10^{-3}$. There is no correlation between the partition coefficients and permeability coefficients for these compounds, which might indicate that permeation of hydrophilic compounds with ether-water partition coefficients very much less than unity does not depend upon the lipophilicity of the compound.

The mechanism of permeation of such hydrophilic compounds through

Table 4-10 The permeability coefficients and ether-water partition coefficients of glucose, urea, glycerol and thiourea, a homologous series of alkanols and hydrocortisone and its 21-n-alkyl esters (see figure 4-9)

Compound	Permeability coefficient (cm/hr)		Ether-water partition coefficient	
Glucose	$9,5 \times 10^{-5}$	1	$8,7 \times 10^{-6}$	1
Urea	$1,2 \times 10^{-4}$	1	$3,2 \times 10^{-4}$	1
Glycerol	$1,4 \times 10^{-4}$	1	$5,1 \times 10^{-4}$	1
Thiourea	$9,6 \times 10^{-5}$	1	$7,3 \times 10^{-3}$	1
Methanol	$2,6 \times 10^{-3}$	2	$9,6 \times 10^{-2}$	3
Ethanol	$4,8 \times 10^{-3}$	2	$2,8 \times 10^{-1}$	3
Propanol	$5,4 \times 10^{-3}$	2	$9,4 \times 10^{-1}$	3
Butanol	$1,5 \times 10^{-2}$	2	5,9	3
Pentanol	$2,2 \times 10^{-2}$	2	$1,6 \times 10^{1}$	3
Hexanol	$4,8 \times 10^{-2}$	2	$6,3 \times 10^{1}$	3
Heptanol	$9,3 \times 10^{-2}$	2	$2,4 \times 10^{2}$	4
Octanol	$9,7 \times 10^{-2}$	2	$8,9 \times 10^{2}$	4
Hydrocortisone	$1,6 \times 10^{-4}$	5	1,4	1
Hydrocortisone-21-acetate	$1,7 \times 10^{-3}$	5	$2,7 \times 10^{1}$	1
" -21-propionate	$9,8 \times 10^{-3}$	5	$9,5 \times 10^{1}$	1
" -21-butyrate	$4,3 \times 10^{-2}$	5	$3,0 \times 10^{2}$	1
" -21-pentanoate	$7,1 \times 10^{-2}$	5	$9,1 \times 10^{2}$	1
" -21-hexanoate	$2,0 \times 10^{-1}$	5	$4,1 \times 10^{3}$	1
" -21-heptanoate	$3,4 \times 10^{-1}$	5	$1,4 \times 10^{4}$	1

[1] Experimentally obtained in this study - first six hours of the experiment.
[2] Taken from Dürrheim (1977:118).
[3] Taken from literature values as detailed in table 4-1 (Hansch & Leo, 1979).
[4] Calculated from the literature values mentioned under 3.
[5] Taken from Smith (1982:127) - (values obtained without the enzyme inhibitor).

hairless mouse skin might therefore be mainly through the water-filled intercellular channels in the stratum corneum.

For the alkanols, the permeability coefficients seem to level off on going below a partition coefficient of 1 on the scale in figure 4-9. This corresponds to a lower limit permeability coefficient for the alkanol series of about $5,4 \times 10^{-3}$ cm/hr.

It seems from figure 4-9 that there is a lower limit to permeability coefficients for hairless mouse skin. This agrees with the suggestion made by Scheuplein and Blank (1973) that there is a finite lower limit of approximately 10^{-6} cm/hr for human skin permeability coefficients. There seems to be an upper limit at approximately 0,5 cm/hr, which corresponds roughly to the permeability coefficients found for different compounds through the dermis only of hairless mouse skin (Dürrheim, 1977:134; Smith, 1982:144).

It is significant to note that for the compounds mentioned in figure 4-9 the ether-water partition coefficient changes with approximately ten logarithmic orders while the permeability coefficients changes only four logarithmic orders. Thus π for partitioning into the lipids of the stratum corneum may be the same for both the alkanols and the hydrocortisone ester series but the π for permeation may deviate markedly from this value (Smith, 1982:144). That means that different diffusional pathways exist for compounds of different polarities and that lipophilicity is not the only determinant of diffusivity and rate of permeation. Consequently it can be concluded that the skin does not act as a simple lipoidal barrier with regard to compounds ranging much in hydrophobicity.

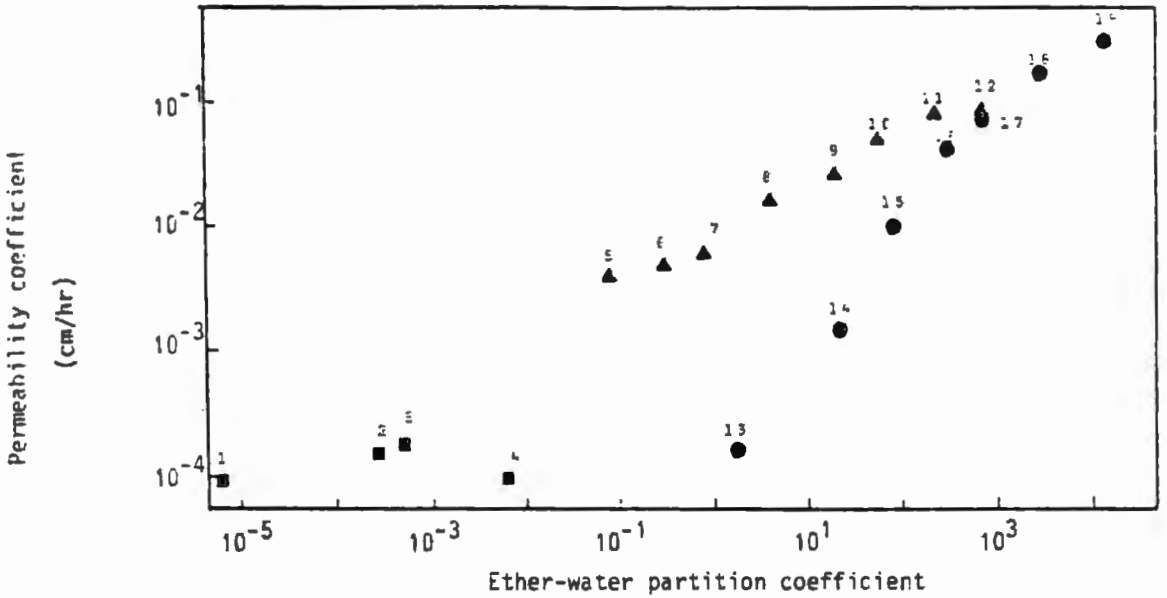

Figure 4-9 The permeability coefficient (cm hr^{-1}) for hairless mouse skin as a function of the ether-water partition coefficient on a log-log scale for the following compounds: (1) Glucose,(2) urea,(3) glycerol,(4) thiourea,(5-12) n-alkanols, methanol to n-octanol,(13) hydrocortisone,(14-19) hydrocortisone 21-n-alkyl esters, -acetate to -heptanoate (see table 4-10).

This discussion of the relationship between ether-water partitioning and permeability was based on the permeability coefficients calculated from data obtained over the first six hours of an experiment. But as mentioned earlier, the permeation of these hydrophilic compounds increased gradually as a function of time - a phenomenon that was not mentioned in literature on the permeation of these compounds through oral mucosa (Siegel, 1981:139) or rabbit skin (Treherne, 1956:171). It was therefore decided to study the causes of this phenomenon more extensively with urea. Consequently one should be cautious about drawing extensive conclusions on the ether-water partioning/percutaneous absorption relationships while there is uncertainty about the permeability coefficient that should be used.

CHAPTER 5 PERCUTANEOUS ABSORPTION OF UREA

5.1 INTRODUCTION

While assessing the permeability coefficient of hydrophilic compounds in the preliminary study (Chapter 4) an increase in permeability as a function of time was noted (see 4.3.3 and 4.3.4 for the detailed results and discussion).

The main objective of this study was to systematically investigate different variables in order to explain the reason for this phenomenon. The increase in permeability may be affected by any one or a combination of the following aspects: The permeating substances (urea and normal saline) may alter the diffusion pathways in the skin mechanistically; the extensive contact with the stirred bathing media may lead to physical deterioration of the skin, since water-soluble components may be washed out of the skin and still another possibility may be that the extensive hydration of the skin makes it more susceptible to microbial attact. It has been reported in the literature that even extensive hydration of the stratum corneum does not impair its barrier properties (Scheuplein and Blank, 1971:716; Behl et al., 1980:346). Therefore physical deterioration and microbial attact were not primary concerns especially because this increasing permeability was even noticeable over the first six hours of an experiment. It was therefore decided to concentrate initially on the effects the permeating substances might have on the permeation pattern.

Urea was chosen for doing a more intensive study on the permeability patterns in relation to other parameters because:

- reproducible results were obtained in the preliminary study with urea;
- the increase in permeability as a function of time was the most pronounced in the case of urea in the preliminary study; and
- urea is used in many preparations for application to the skin, so that its effects on skin are interesting in their own right.

By studying the effect of the following variables on the permeation of urea through full thickness hairless mouse skin, one should be able to clarify the reasons involved in the observed phenomenon:

The time before steady state is reached;

the influence of hydration by measuring permeation after different prehydration times and by doing sequential permeation studies on the same piece of skin;

the influence of the isotonic bathing media by using normal saline, MOPS and TRIS at a pH of approximately 5,5 ;

the influence of the concentration of the permeating compound by using three different concentrations;

the effect of urea on the permeation of water and methanol by means of dual label experiments; and

the effect of stripping on the permeation of urea.

All the permeation studies were done in vitro by measuring the

permeation of urea through hairless mouse skin by means of a diffusion cell system as described under 4.3.2.3.

5.2 ASSESSMENT OF THE TIME TO REACH STEADY STATE

5.2.1 INTRODUCTION

While assessing the permeability of the hydrophilic compounds in the preliminary study it became evident that a steady state could not be obtained with urea within 30 hours; the permeation profile was still exhibiting logarithmic increases in permeation rate as a function of time at that stage.

Based on work with other compounds the permeation rate maximum could be no larger than about 0,3 cm/hr (Dürrheim, 1977:134), which represents the maximum rate found for permeation through the dermis.

5.2.2 EXPERIMENTAL

5.2.2.1 MATERIALS

(^{14}C)-Urea[1] in a concentration of 3,5 x 10^{-5} M was used.

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[1] New England Nuclear, 549 Albany Street, Boston, Massachusetts.

5.2.2.2 PROCEDURE

The general procedure as described under 4.3.2.3 was followed. An isotonic solution of 0,01 M unlabelled urea[2] in normal saline[3] was placed in both donor and receiver cells for the reasons mentioned under 4.2.1.1. A long continuous experiment was executed and the cell contents were completely replaced every eight hours. Donor cell samples were only taken just before and after replacement of donor cell contents. Receiver cell samples were taken at 2-4 hourly intervals except overnight when only one receiver sample was taken in the morning before the replacement of the contents of both cells.

Since these studies on the permeation of urea were done in Potchefstroom while the preliminary studies were done in Ann Arbor, USA, the following items used in the analysis of samples were different: scintillation vials[4], scintillation coctail[5] and the liquid scintillation counter[6].

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[2] Analar, Packed by G.D Searle, Johannesburg.

[3] Labethica, Sodium chloride 0,9% w/v pH app. 5,5, sterile pyrogen-free, for intravenous use, Bethlehem.

[4] 20 ml Glass vials from Beckman Instruments, Johannesburg.

[5] Aquagel from Chemlab, Sandton.

[6] TRI CARB 460C Liquid Scintillation System, Packard Instrument Co., Illinois.

The rest of the procedures and apparatus were identical to that used in the USA.

The hairless mice[7] were a different species from those used in the USA.

5.2.3 RESULTS

Table 5-1 and figure 5-1 give the relevant data on the time it took to obtain a steady state permeation rate of urea through full thickness hairless mouse skin.

5.2.4 DISCUSSION AND CONCLUSIONS

When the permeability coefficient is observed as a function of time a rapid increase in permeability is seen over the first 96 hours. Between 96 and 123 hours a steady state permeation pattern is observed. This is in contrast to the results other workers obtained for the percutaneous absorption of other hydrophilic compounds like water, methanol and ethanol (Ireherne, 1956:171; Dürrheim et al., 1980:781) where a steady state was reached within an hour after the experiment started.

In this permeation study with urea it seems as if the epidermal barrier had undergone changes in the diffusion process with time.

---

[7] Nude mutant gene from genetic background BALB/c x HA/Icr, Dairy Research Council, Irene.

Table 5-1 The permeability coefficient of urea at different time intervals for full thickness hairless mouse skin over a period of 123 hours

Time (hours)	Permeability coefficient x 10^3 cm/hr	Standard deviation [1]
11	0,3	0,2 (4)
21	1,4	1,4 (4)
25	5,1	4,9 (4)
43	23,8	9,5 (4)
51	86,6	42,3 (4)
74	115,9	60,7 (4)
96	199,6	91,2 (4)
123	198,0	45,8 (4)

[1] Number of determinations in brackets.

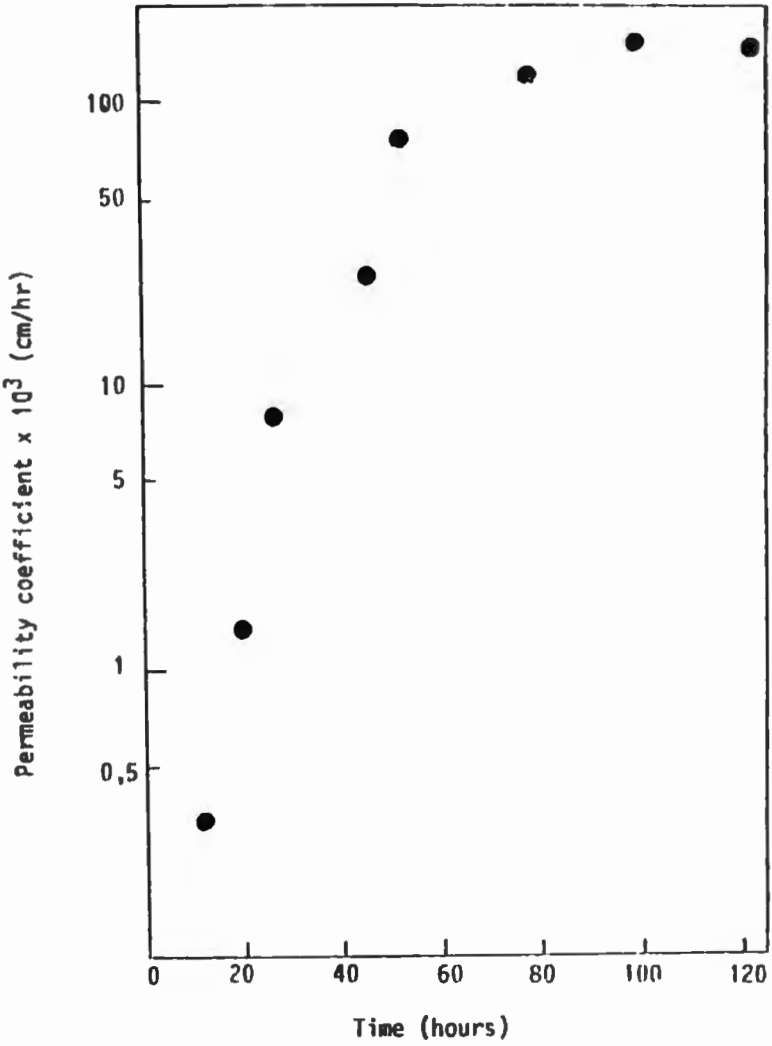

Figure 5-1 The permeability coefficients of a 0,01 M urea solution as a function of time.

The question was whether it was due to the influence of the permeating substance or whether it was a hydration effect that had been observed. The term "hydration" should only be applied to instances where water is bound by components of the skin and particularly the stratum corneum. It will be assumed that hydration does not cause any physical changes of the skin strata involved.

5.3 THE INFLUENCE OF HYDRATION

5.3.1 INTRODUCTION

According to hydration studies done by other workers (Behl et al., 1980:346), hydration was not expected to have a large influence over the first 12-24 hours. The plateau phase was reached in 96 hours in the previous experiments. It was therefore decided to hydrate the skin for different time intervals up to 96 hours before starting a permeation experiment. The objective was to discriminate between the hydration effect and the effect that urea might have had on the skin during permeation.

5.3.2 EXPERIMENTAL

5.3.2.1 MATERIALS

The skin was hydrated with a normal saline solution. A labelled

urea solution of $3,7 \times 10^{-5}$ M was monitored as the permeating substance. During the permeation studies both the donor and receiver cells contained 0,01 M unlabelled urea.

5.3.2.2 PROCEDURE

The same general procedure as described under 4.3.2.3 and 5.2.2.2 was followed. Four pieces of skin was hydrated simultaneously for 24, 48, 72 and 96 hours respectively before the permeation experiment commenced.

5.3.3 RESULTS

The relevant data on the permeability of urea through full thickness hairless mouse skin after the skin has been hydrated for different time intervals are given in table 5-2 and figure 5-2.

5.3.4 DISCUSSION AND CONCLUSIONS

No significant difference in the permeability pattern was found for the four different hydration times. This result indicates that urea did not induce an increase in its own permeability in the concentration used in this experiment. It has to be noted that a 0,01 M of unlabelled urea solution was used on both sides of the membrane during a permeation experiment.

A possible explanation for the observed increase in permeability

Table 5-2 The permeability coefficients of a 0,01 M urea solution after full thickness hairless mouse skin has been hydrated for different time intervals [1]

Time (hours)	Permeability coefficient x 10^3 cm/hr			
	Prehydration time intervals (hours)			
	24	48	72	96
26	10			
29	16			
32	24			
49	36	45		
54	57	64		
72	48	105	66	
78	78	148	97	
102	108	148	166	115
118	179	162	172	124
119	123	183	219	173

[1] Four pieces of skin from the same mouse were used.

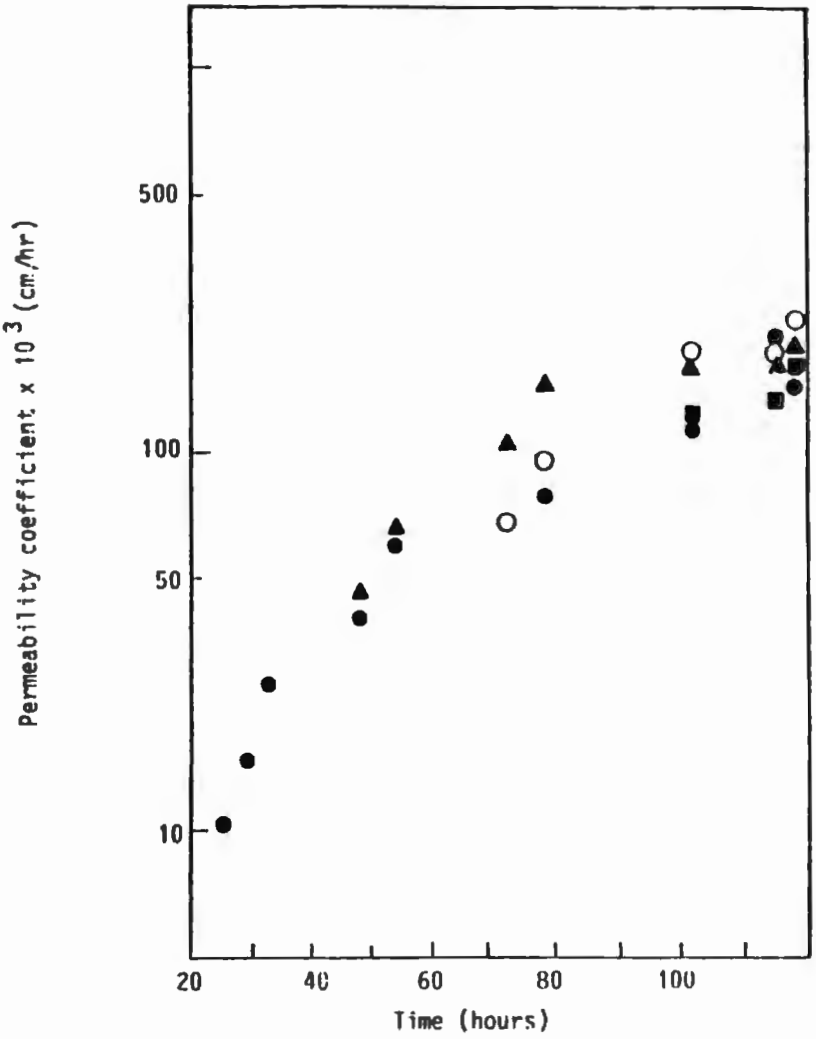

Figure 5-2 The permeability coefficients of a 0,01 M solution of urea as a function of time after full thickness hairless mouse skin has been hydrated for 24 hours -●-, 48 hours -▲-, 72 hours -○- and 96 hours -■-. Four pieces of skin from the same mouse were used.

with time may be the effect of the prolonged contact of normal saline with the permeation barrier.

5.4 SEQUENTIAL RUN PROCEDURE

5.4.1 INTRODUCTION

The objective of this study was to do an experiment comparable with those done by other workers (Behl et al., 1980:346) as a control of the effect of hydration.

5.4.2 EXPERIMENTAL

5.4.2.1 MATERIALS

Between runs the skin was rinsed and soaked with normal saline. Each run was done with a $3,7 \times 10^{-5}$ M solution of labelled urea. A 0,01 M urea solution in saline was used as the diffusional medium.

5.4.2.2 PROCEDURE

The same general procedure as described under 4.3.2.3 and 5.2.2.2 was followed, except that after each run the contents of both diffusion cells was removed and both cells were rinsed

five times with normal saline. Then each cell was filled with 1,2 ml normal saline and left for approximately 12 hours. Each cell was rinsed another five times before the next run commenced. Control samples of the rinsing and soaking solutions after different time intervals showed that this procedure was efficient in eliminating labelled urea from the previous run in each case. The saline rinses contained no urea and no urea was present between permeation experiments.

5.4.3 RESULTS

See table 5-3 and figure 5-3 for the relevant data on the permeability of urea with the sequential run procedure. Each run is represented by two points. The first point is the mean permeability over the first four hours of each run and the second point is the mean permeability over the last four hours of each run.

5.4.4 DISCUSSION AND CONCLUSIONS

Although urea was not continuously in contact with the skin during the sequential run procedure, the same pattern of increasing permeability as with the continuous experiments was found (see figure 5-1). As with the previous experiment the results show that it is not the prolonged contact of urea with the skin that alters the permeability of the barrier, but some other common factor present in both the continuous and sequential run procedures. A normal saline solution was continuously in contact with the skin in both procedures. This suggests that hydration may be

Table 5-3 The permeability coefficients of a 0,01 M urea solution when the sequential run procedure was followed over a period of 128 hours

Time (hours)	Permeability coefficient x 10^3 cm/hr	Standard deviation [1]
2	0,08	0,02 (4)
6	0,2	0,1 (4)
22	1,0	0,1 (4)
26	2,6	2,7 (4)
32	3,7	2,7 (4)
35	7,7	6,7 (4)
50	11,5	2,3 (4)
54	19,1	7,8 (4)
70	48,6	28,6 (4)
74	54,9	28,0 (4)
96	95,0	52,2 (4)
100	90,0	34,0 (4)
123	98,0	43,0 (4)
128	94,0	26,0 (4)

[1] Number of determinations in brackets.

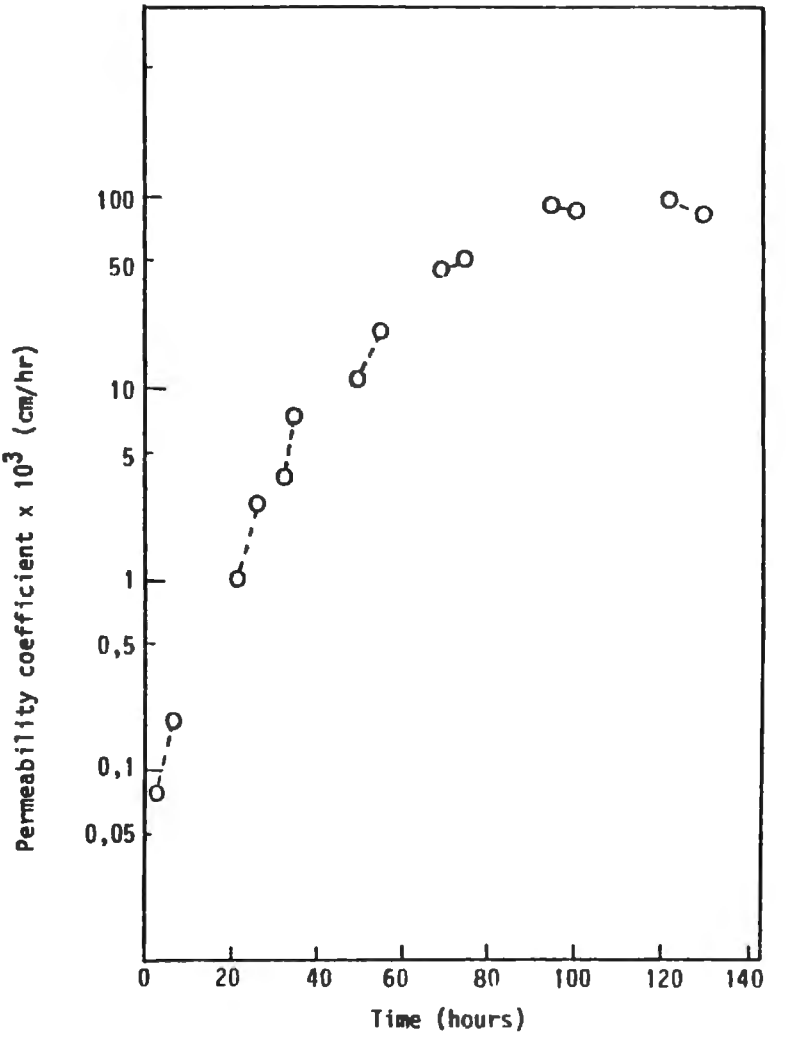

Figure 5-3 The permeability coefficient of a 0,01 M urea solution as a function of time when the sequential run procedure is followed. Each O--O represents the mean permeability coefficient of the first four hours and the last four hours of each sequential run.

the cause of the increasing permeability.

Other workers (Behl et al., 1980:350) did not observe such a dramatic effect of hydration with the permeation of a series of alkanols over a permeation period of 30 hours. That might be an indication that the hydration factor particularly affects the barrier for urea permeation but not that of semi-polar alkanols, or that the effect of hydration was not so acutely visible over a time span of 30 hours.

It might also be that urea has a measurable effect on its own permeation in which case one would expect that effect to increase with an increase in the concentration of urea in contact with the skin.

5.5 DIFFERENT CONCENTRATIONS OF UREA

5.5.1 INTRODUCTION

The objective of this study was to assess the effect of concentration and hypertonicity on the percutaneous absorption of urea through full thickness hairless mouse skin.

The concentration of urea that is most often used in applications to the skin is 10% w/v. In vivo permeation studies have also been done with different formulations containing 10% w/v of urea (Wohlrab and Hassler, 1981:277).

For in vitro permeation studies usually very low concentrations of labelled compounds are used for various reasons, e.g. very low concentrations can be monitored in in vitro systems; with very low concentrations the activity coefficient of the diffusing substance is substantially linear to the concentration, and with the high cost of labelled compounds, it is economically convenient to use low concentrations. The lowest concentration of urea used in this study was 0,01 M.

A 10% w/v solution of urea is equivalent to a 1,67 M solution. Consequently it was decided to utilise the 0,01 M and 1,67 M solutions as the minimum and maximum concentrations repectively. As a third concentration a 0,27 M solution, which is the maximum concentration of urea that is still isotonic, was chosen.

With 2 logarithmic orders of magnitude difference between the minimum and maximum concentrations, a difference in the permeation pattern of urea was expected if any concentration dependency exists.

Since the 1,67 M solution of urea is also very hypertonic (1670 millimoles per litre instead of 270 millimoles per litre which is isotonic), effects of hypertonicity on the permeation pattern of urea might also be evident.

5.5.2 EXPERIMENTAL

5.5.2.1 MATERIALS

The three different concentrations were made up of labelled and

unlabelled urea. Each contained the same amount of labelled urea ($3,7 \times 10^{-5}$ M) with unlabelled urea added to obtain the desired concentration in the donor cell. The receiver cell contained a 0,01 M solution of unlabelled urea.

5.5.2.2 PROCEDURE

The same general procedure as described under 4.3.2.3 and 5.2.2.2 was followed.

5.5.3 RESULTS

See table 5-4 and figure 5-4 for the relevant data on the permeability of different concentrations of urea.

5.5.4 DISCUSSION AND CONCLUSIONS

When the experimental variation is taken into account the same quantitative and qualitative permeation pattern is seen with all three concentrations of urea although the highest concentration differed from the lowest concentration by 2 logarithmic orders of magnitude. The increasing permeability pattern definitely does not depend upon the concentration of the permeating urea in the concentration range of 0,01 M to 1,67 M.

Table 5-4 The permeability coefficients of different concentrations of urea through full thickness hairless mouse skin over a period of 100 hours

Time	Permeability coefficient x 10^3 cm/hr [1]					
(hours)	0,01 M		0,27 M		1,67 M	
2					0,07	(0,03)
7			0,6	(0,1)		
9					1,5	(1,8)
11	0,3	(0,2)				
21	1,4	(1,4)				
25	7	(5)	4	(1)	2,1	(0,4)
30					2,8	(2,7)
43	24	(10)	20	(12)		
48					21	(4)
51	87	(42)				
55					98	(12)
60			47	(16)		
74	116	(61)				
78			90	(55)	54	(15)
96	200	(91)				
101			96	(54)	96	(10)

[1] Standard deviation in brackets. Four determinations were made in each case.

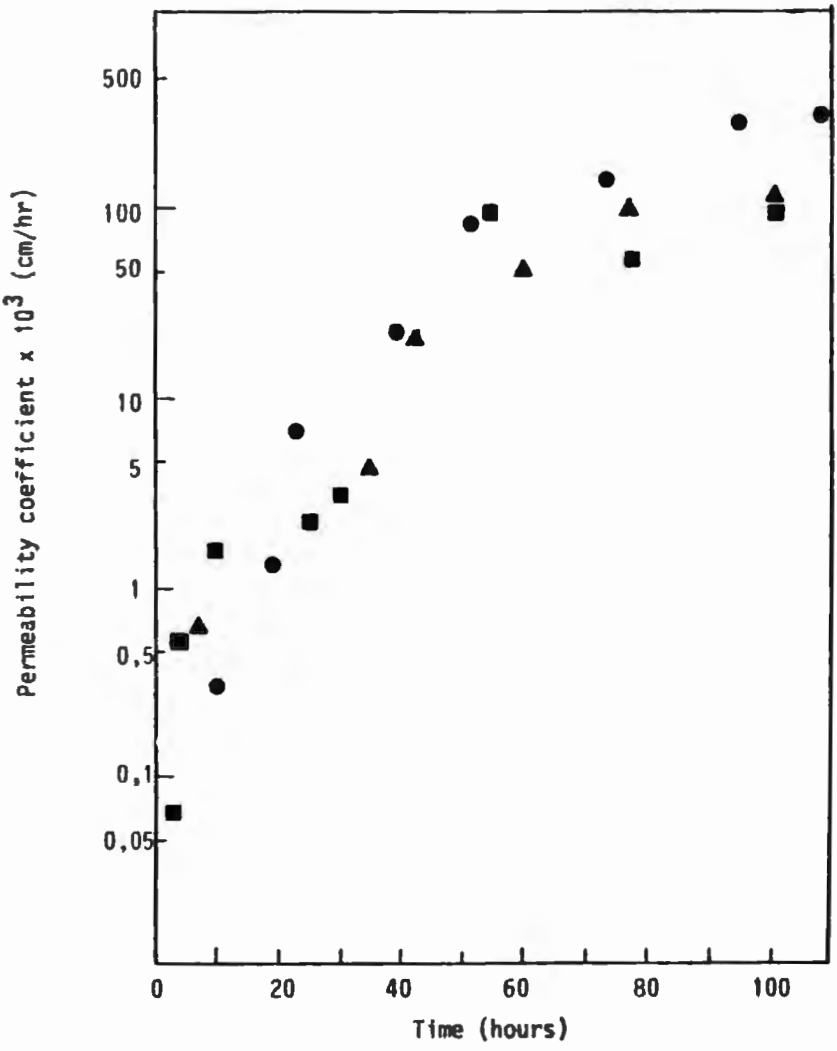

Figure 5-4 The permeability coefficients of different concentrations of urea as a function of time, 0,01 M -●- ; 0,27 M -▲- ; 1,67 M -■- .

The only other compound except water that might influence the permeability barrier is the sodium chloride used to render the bathing medium isotonic. Comparing the effect of different isotonic bathing media on the permeation of urea through full thickness hairless mouse skin should give an indication of the effect of sodium chloride on the permeability barrier.

5.6 PERMEATION OF UREA IN THE PRESENCE OF DIFFERENT ISOTONIC MEDIA

5.6.1 INTRODUCTION

The objective was to assess the effect of different isotonic bathing media on the permeation of urea through full thickness hairless mouse skin. 3-(N-Morpholino) propanesulphonic acid (MOPS)[8] and Tris (hydroxyme) aminomethane (TRIS)[9] which are both well-known biological buffers, were chosen to compare to the normal saline as a bathing medium.

---

[8] BDH Chemicals Ltd., Distributed by Hickman & Kleber (Pty) Ltd. Johannesburg.

[9] Merck Chemicals (Pty) Ltd., Johannesburg.

5.6.2 EXPERIMENTAL

5.6.2.1 MATERIALS

MOPS and TRIS were used in a concentration of 0,05 M. The pH of the solutions was adjusted to 5,5 $\pm$ 0,2 by the addition of 1 M NaOH and 1 M HCl respectively. Urea was used in a concentration of 0,27 M.

5.6.2.2 PROCEDURE

The procedure was exactly the same as that used with the sodium chloride solution as described under 4.3.2.3 and 5.2.2.2.

5.6.3 RESULTS

Please see table 5-5 and figure 5-5 for the relevant data on the permeation of urea in the presence of different isotonic media.

5.6.4 DISCUSSION AND CONCLUSIONS

When the experimental variation is taken into account the permeation of urea in the presence of MOPS and TRIS buffers follows the same pattern of increasing permeation as in the presence of normal saline.

Table 5-5 The permeability coefficients of a 0,01 M solution of urea in the presence of three different isotonic media (pH 5,5±0,2) over a period of 96 hours

Time	Permeability coefficient x 10^3 cm/hr			
(hours)	Normal saline [1]		MOPS(0,05 M) [2]	TRIS(0,05 M) [2]
4			0,6	1
11	0,3	(0,2)		
20	1,4	(1,4)	0,8	7
25	7	(5)		
28			1,2	4
44	24	(10)	10	
51	87	(42)		
57			16	39
70			49	69
74	116	(61)		
92			67	34
96	200	(91)		

[1] Standard deviation in brackets. Four determinations were made in each case.

[2] The mean of two determinations.

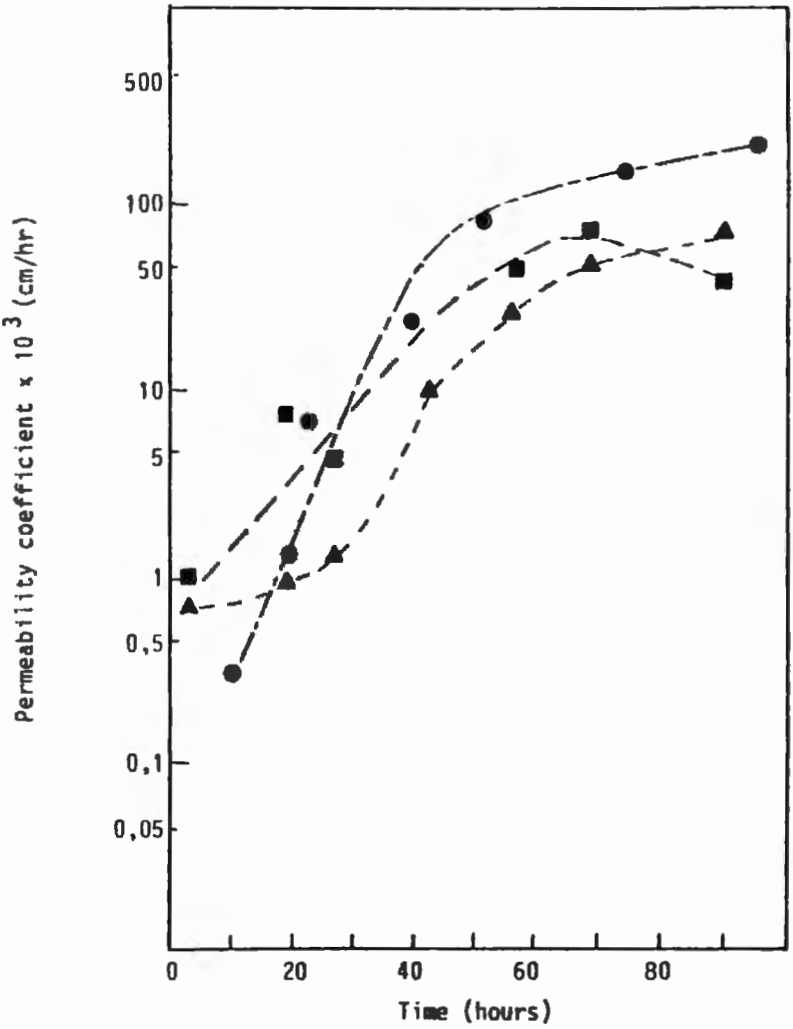

Figure 5-5 The permeability coefficients of a 0,01 M solution of urea in different isotonic media as a function of time, normal saline -●- ; MOPS -▲- ; TRIS -■- .

The permeability coefficient at 70-78 hours differed quantitatively for the three isotonic media and although the limited data prohibit a definite conclusion it seems as if the permeation of urea in the presence of MOPS and TRIS is a little slower in the steady state phase than is the case in the presence of normal saline. This small variation may also be ascribed to mouse-to-mouse variation.

5.7 THE SIMULTANEOUS PERMEATION OF ^{14}C-UREA AND ^{3}H-WATER

5.7.1 INTRODUCTION

The permeability pattern of urea as found in this study differs very much from that of the reported pattern of other hydrophilic compounds like water, methanol and ethanol. In the studies with water, methanol and ethanol the increase in permeability as a function of time over such a prolonged period was not observed and the steady state phase was attained within one hour. If these differences are assosiated in some way with the heterogeneous nature of the stratum corneum, one would expect that in a dual-label experiment where ^{14}C-urea and ^{3}H-water are permeated simultaneously that urea will show the increasing permeability pattern while the permeability of water will not change as a function of time. In the following study this hypothesis was tested.

5.7.2 EXPERIMENTAL

5.7.2.1 MATERIALS

The donor cell contained 0,01 M unlabelled urea, $3,5 \times 10^{-5}$ M ^{14}C-urea and $5,6 \times 10^{-5}$ M ^{3}H-water. The receiver cell contained 0,01 M unlabelled urea. Both solutions were made isotonic with normal saline.

5.7.2.2 PROCEDURE

The general procedure as described under 4.3.2.3 was followed. Samples were taken every hour from the receiver cell for four hours and samples from the donor cell were taken before and after each four-hour run. These four-hour runs were repeated at 24, 28, 72, 96 and 144 hours.

5.7.3 RESULTS

Table 5-6 and figure 5-6 display the relevant data on the simultaneous permeation of ^{14}C-urea and ^{3}H-water.

5.7.4 DISCUSSION AND CONCLUSIONS

The water and the urea exhibit the same pattern of rapid increase in permeability over 150 hours. This observation leads to the

Table 5-6 The permeability coefficients for the simultaneous permeation of ^{14}C-urea and ^{3}H-water through full thickness hairless mouse skin over a period of 145 hours

Time	Permeability coefficient x 10^3 cm/hr [1]	
(hours)	^{14}C-urea	^{3}H-water
2	1 (1) (4)	2 (1) (4)
6	4 (2) (4)	6 (2) (4)
26	11 (7) (4)	20 (11) (4)
50	25 (24) (4)	41 (28) (4)
73	43 (26) (4)	67 (24) (4)
104	75 (28) (4)	106 (57) (4)
145	153 (91) (4)	252(157) (4)

[1] Standard deviation in the first brackets and the number of determinations in the second brackets.

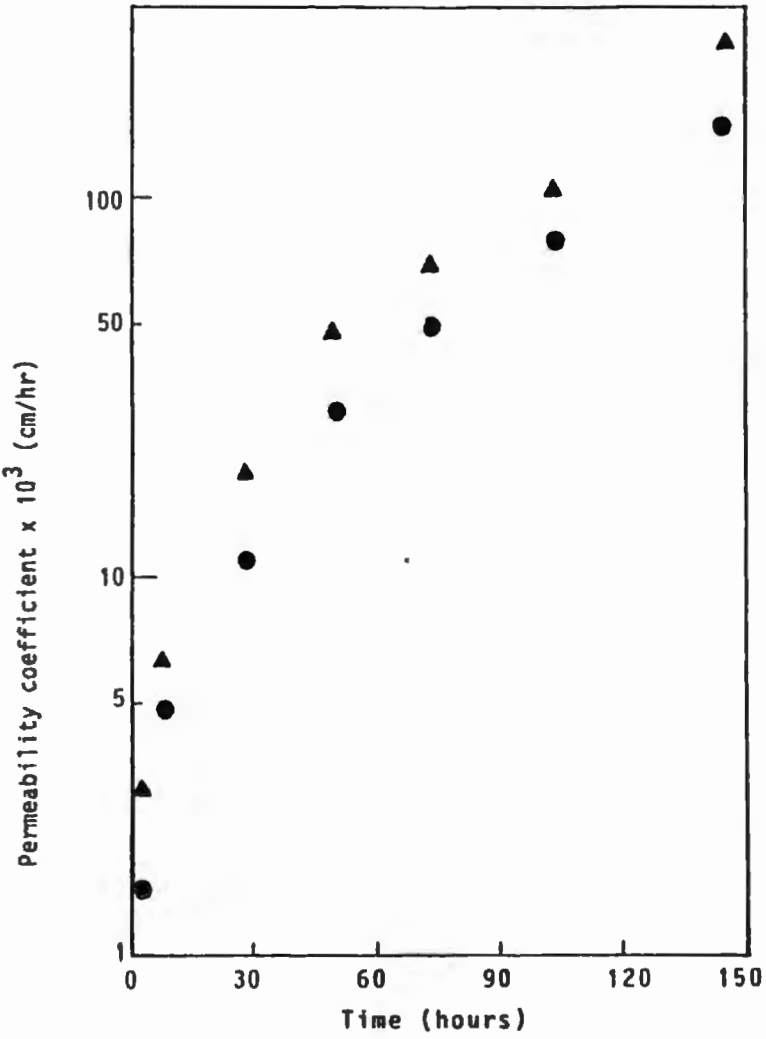

Figure 5-6 The permeability coefficient of a 0,01 M urea (●) and a 5,6 x 10^{-5} M ^{3}H-water solution (▲) for full thickness hairless mouse skin as a function of time.

conclusion that there is no significant difference between the effect of the barriers to the permeation of these compounds. In fact it seems to be the same barrier operating for both compounds.

5.8 THE SIMULTANEOUS PERMEATION OF ^{14}C-METHANOL AND ^{3}H-WATER

5.8.1 INTRODUCTION

According to Behl et al. (1980:346) the permeabilities of the hydrophilic solutes, water, methanol and ethanol were not changed by hydration when using hairless mouse skin (SKH-hr^{-1} strain). In contrast to the hairless mouse skin results, Behl et al. found that hydration studies with the Swiss mouse revealed hydration effects on the permeability of water, methanol and ethanol. The permeability of water increased up to 30 hours of hydration and showed signs of levelling off between 30 and 43 hours. The hydration profile differences between the Swiss mouse and the hairless mouse were apparently due to the abundant follicular presence in the Swiss species (Behl et al. 1980:346).

Qualitatively the same permeation profile was found for urea with the SKH-hr^{-1} hairless mouse skin and the South African counterpart (nude mutant gene from genetic background BALB/c x IIA/Icr) - see table 5-7 and figure 5-7. Although the latter also has an abundance of follicles, with both species an increase in permeation as a function of time was observed. From this observation it can be concluded that the hair follicles are not the major determinat of the accelerated permeation.

Table 5-7 The permeability coefficients of a 0,01 M solution of urea for different species of mice through full thickness skin over a period of 50 hours

Time	Permeability coefficient x 10^3 cm/hr [1]	
(hours)	SKH-hr^{-1}	BALB/c x HA/Icr
1		0,2 (0,2) (4)
2	0,1 (0,2) (2)	
4		0,5 (0,6) (4)
8	0,5 (0,8) (2)	
9		1,2 (1,4) (4)
12	1,8 (2,8) (2)	
22	11,8 (11) (2)	
25		6,9 (5,1) (4)
48		23,6 (0,3) (4)

[1] Standard deviation in the first brackets and the number of determinations in the second brackets.

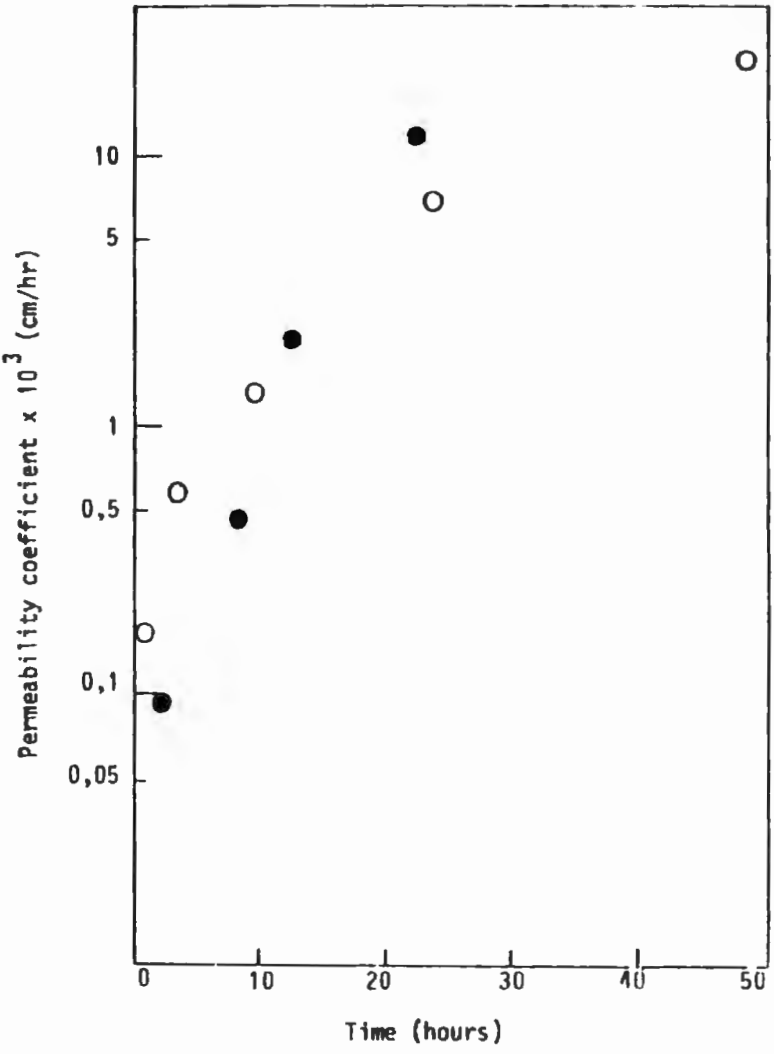

Figure 5-7 The permeability coefficients of a 0,01 M solution of urea for different species of hairless mice as a function of time. SKH-hr^{-1} - ● - ; BALB/c x HA/Icr − O− .

Since the stratum corneum is generally accepted as the major barrier to the permeation of hydrophilic compounds (Treherne, 1956: Scheuplein & Blank, 1971:704; Wohlrab and Hassler, 1981:277), the increase in permeation must be a function of changes in the stratum corneum. The question is if these changes have the same effect on the permeation of all hydrophilic compounds. From the results found when water and urea were permeated simultaneously it seemed as if the changes in the stratum corneum affected the permeation of water and urea in the same way.

This study was conducted to test the hypothesis that the mechanism of permeation of urea and methanol differs. Unfortunately ^{3}H-methanol was not available at the time of the study, but only ^{14}C-methanol. Therefore a simultaneous run of ^{14}C-methanol and ^{3}H-water was to be compared to the simultaneous run of ^{14}C-urea and ^{3}H-water.

5.8.2 EXPERIMENTAL

5.8.2.1 MATERIALS

The donor cell contained $7,5 \times 10^{-4}$ M ^{14}C-methanol and $5,6 \times 10^{-5}$M ^{3}H-water in a normal saline solution. The receiver cell contained only normal saline solution.

5.8.2.2 PROCEDURE

Exactly the same procedure as described under 5.8.2.2 was followed.

5.8.3 RESULTS

Table 5-8 and figure 5-8 display the relevant data on the simultaneous permeation of ^{14}C-methanol and ^{3}H-water.

5.8.4 DISCUSSION AND CONCLUSIONS

The simultaneous permeation of methanol and water also exhibits a pattern of increasing permeation, which correlates very well with the trend observed when urea and water permeated simultaneously.

The results obtained when urea and water permeated simultaneously exhibit a slight quantitative difference from those obtained with methanol and water. But when experimental variation is taken into account, the difference cannot be ascribed to mechanistic differences.

When the permeation patterns over the first six hours of these experiments are compared, it is clear that urea, methanol and water exhibit the same increasing permeation pattern (see figures 5-9 and 5-10).

Concequently it can be concluded that no significant difference

Table 5-8 The permeability coefficients for the simultaneous permeation of ^{14}C-methanol and ^{3}H-water through full thickness hairless mouse skin over a period of 127 hours

Time (hours)	Permeability coefficient x 10^3 cm/hr [1]					
	^{14}C-methanol			^{3}H-water		
2	2	(1)	(4)	4	(1)	(4)
5	8	(3)	(4)	16	(4)	(4)
25	17	(5)	(4)	28	(8)	(4)
50				102	(36)	(4)
73	91	(24)	(4)	190	(52)	(4)
96	79	(32)	(4)	173	(56)	(4)
127	105	(65)	(4)	168	(47)	(4)

[1] Standard deviation in the first brackets and number of determinations in the second brackets.

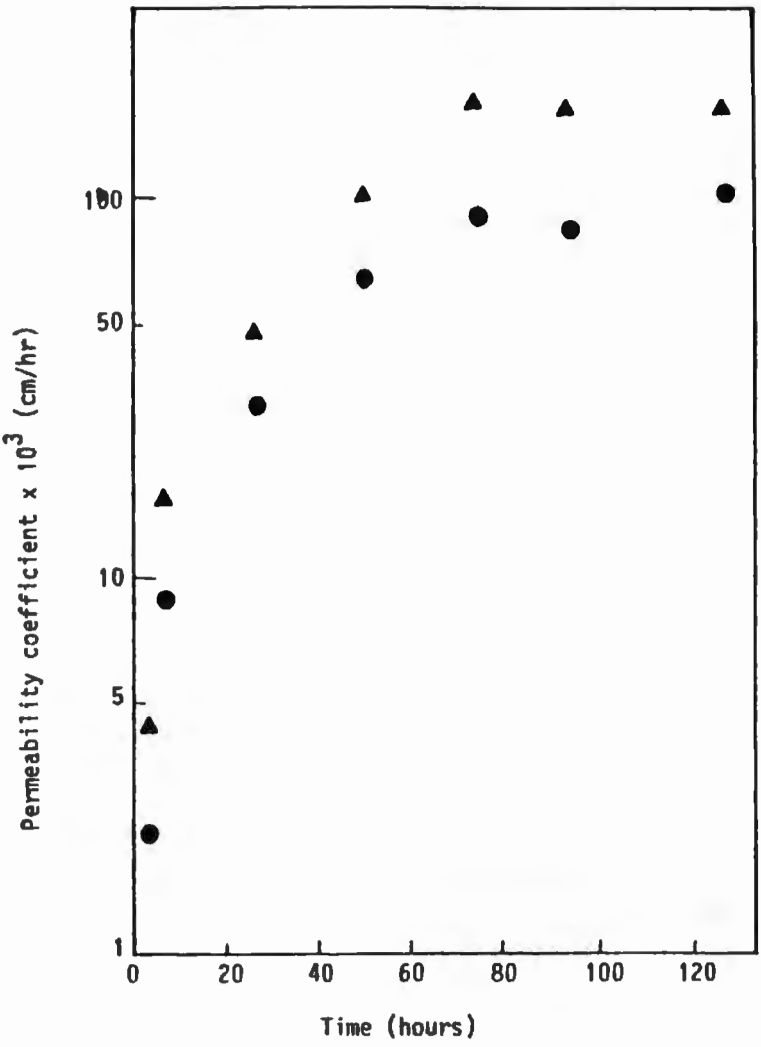

Figure 5-8 The permeability coefficient of a $7{,}5 \times 10^{-4}$M ^{14}C-methanol (●) and a $5{,}6 \times 10^{-5}$M ^{3}H-water solution (▲) as a function of time for full thickness hairless mouse skin.

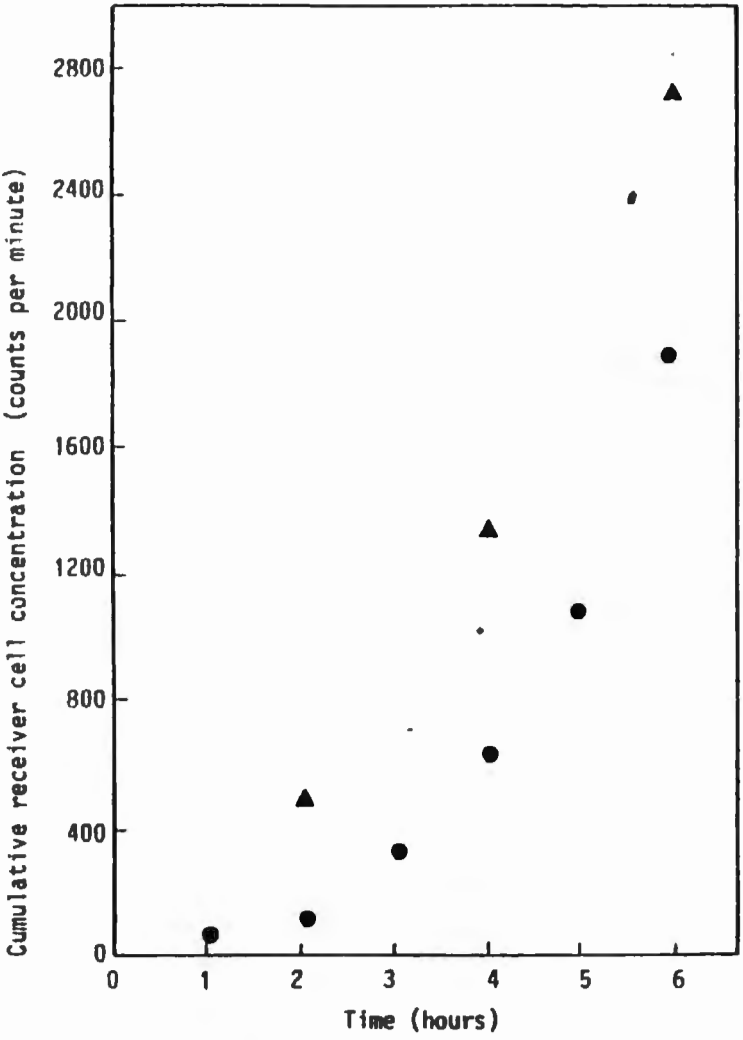

Figure 5-9 The cumulative receiver cell concentration as a function of time for methanol (●) and urea (▲) over the first six hours of an experiment.

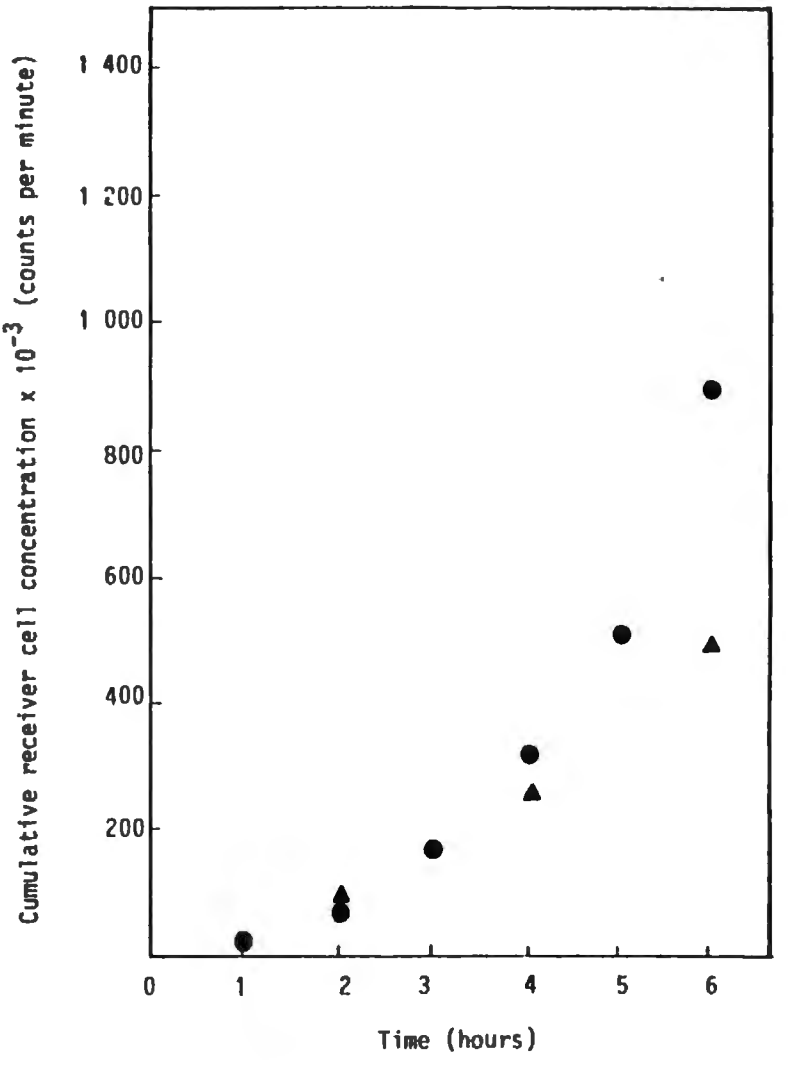

Figure 5-10 The cumulative receiver cell concentration as a function of time for water in the presence of methanol (●) and water in the presence of urea (▲) over the first six hours of an experiment.

in the mechanism of and the barrier to the permeation of urea, methanol and water could be found.

5.9 THE PERMEATION OF UREA THROUGH STRIPPED SKIN

5.9.1 INTRODUCTION

Although the stratum corneum is generally accepted as the main barrier to the permeation of substances through skin, it is also well known that hydration of the stratum corneum leads to an increase in permeability. When the stratum corneum becomes so much hydrated that if offers no significant barrier to the permeation of very hydrophilic compounds, the remainder of the epidermis might become the major barrier to the permeation of hydrophilic compounds through excessively hydrated skin.

By stripping full thickness skin to different degrees and conducting exactly the same experiment on the different pieces of stripped skin, a qualitative estimation of the relative contributions of other cell layers to the barrier properties should be possible.

5.9.2 EXPERIMENTAL

5.9.2.1 MATERIALS

The hairless mouse skin was stripped by means of Scotch Magic Tape

(1R No 810, 3M South Africa (Pty) Ltd. Tel. 011-36 3211). The donor cell contained 0,01 M unlabelled urea and $3,7 \times 10^{-5}$ M ^{14}C-urea and the receiver cell contained 0,01 M unlabelled urea. Both solutions were made isotonic with normal saline.

5.9.2.2 PROCEDURE

Stripping was performed by applying succesive pieces of tape to the abdominal region of a freshly killed mouse, making sure that the areas to be exposed for diffusion were covered.

The procedures during the permeation study were the same as described under 4.3.2.3

5.9.2 RESULTS

Table 5-9 and figure 5-11 display the relevant data on the permeation of ^{14}C-urea through stripped skin.

5.9.4 DISCUSSION AND CONCLUSIONS

Beyond 20 hours the full thickness hairless mouse skin showed the lowest permeability, followed by the 10 times stripped and 20 times stripped skin respectively (figure 5-11). This phenomenon

Table 5-9 The permeability coefficient for 0,01 M urea permeating full thickness and stripped hairless mouse skin over a period of 124 hours

Time	Permeability coefficient x 10^3 cm/hr [1]			
	Full thickness [2]		Stripped	
(hours)	(Mouse A)	(Mouse B)	10x (Mouse C)	20x (Mouse D)
1	0,2 (0,2) (4)			2,6 (3,3) (3)
3			0,6 (0,5) (4)	
4	0,5 (0,6) (4)			6,0 (7,6) (3)
11		0,3 (0,2) (4)		
19				11 (6,0) (3)
21		1,4 (1,3) (4)		
22			1,1 (0,8) (4)	39 (27) (3)
25	1,5 (0,1) (4)	6,9 (5,2) (4)		
28	2,8 (0,9) (4)		4,4 (2,1) (4)	
43		24 (10) (4)		
46	24 (0,3) (4)			51 (29) (3)
51		87 (42) (4)		
53	103 (10) (4)		19 (0,5) (4)	
71				90 (42) (3)
74		116 (61) (4)		
75	104 (24) (4)		25 (21) (4)	
94	225 (13) (4)	200 (91) (4)	47 (36) (4)	
124		198 (46) (4)	108 (94) (4)	

[1] Standard deviation in the first brackets and the number of determinations in the second brackets.

[2] Two sets of experiments on different mice are presented to illustrate the differences in average values for full thickness skin of different mice.

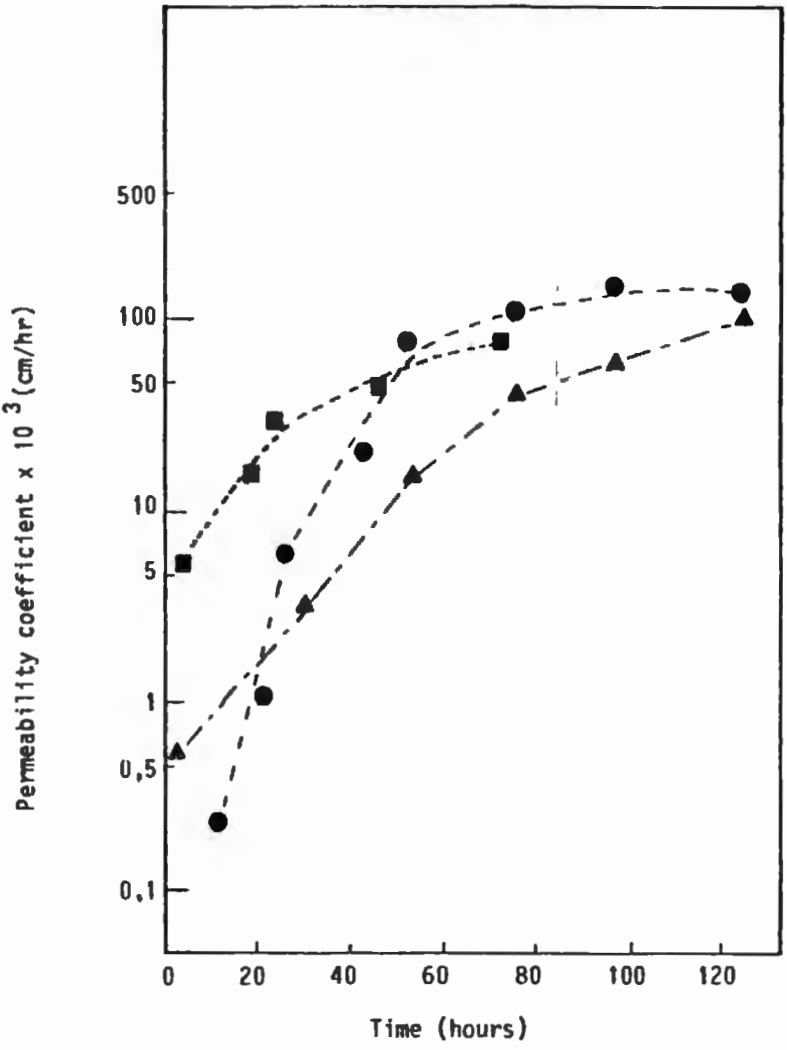

Figure 5-11 The permeability coefficient as a function of time for the permeation of a 0,01 M urea solution through full thickness hairless mouse skin (●), skin that has been stripped 10 times (▲) and 20 times (■).

is expected when the stratum corneum forms the main barrier to permeation. The higher permeability which is observed initially with the 10 times stripped skin could be ascribed to the decrease in the thickness of the horny layer.

Stripping is incomplete but removes the most heavily bacteria contaminated layers. Therefore micro-organisms might be more abundantly present on the full thickness skin than on the stripped skin. The outer loose, more "hydrophilic" cells of the full thickness stratum corneum might form a better growth medium for micro-organisms than the lipophilic surface of the stripped skin during extensive hydration as found during the experiments in this study.

Unexpected and very interesting is that the permeation through full thickness skin seems to increase much faster than permeation through the 10 times stripped skin, so much so that after about 40 hours the permeability coefficients of urea permeating full thickness skin are significantly higher than those of urea permeating the 10 times stripped skin.

5.10 PERMEATION OF UREA IN THE PRESENCE OF L-LEUCINE

5.10.1 INTRODUCTION

If the permeability barrier depends upon the maintenance of normal cell function, the reduced uptake of an essential amino acid like L-leucine should be an indication of the deterioration of the permeability barrier.

Or if the deterioration of the permeability barrier is counteracted by the addition of a substance that will aid the cells in maintaining their function for a longer period, the permeation of urea should reflect that influence.

The objective was to determine if there is a difference in the permeation pattern of urea when an essential amino acid like ℓ-leucine is added as a co-permeant.

5.10.2 EXPERIMENTAL

5.10.2.1 MATERIALS

(^{14}C)-Urea (3,7 x 10^{-5}M), unlabelled urea (0,01 M) and ℓ-(4,5-^{3}H)-leucine[10] (1 x 10^{-10}M) were used with sodium chloride added to render all solutions isotonic.

5.10.2.2 PROCEDURE

The general procedure as described under 4.3.2.3 and 5.2.2.2 was followed.

For the permeation of ℓ-leucine alone, the ℓ-leucine was mixed with normal saline in the donor cell. The receiver cell contained only normal saline.

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[10] Amersham International Ltd., Amersham UK. Batch 89.

...on of urea and ℓ-leucine could be followed simultaneously because they were labelled ^{14}C and ^{3}H respectively. The donor cell contained labelled urea ($3,7 \times 10^{-5}$ M), unlabelled urea (0,01 M) and labelled ℓ-leucine (1×10^{-10} M). The receiver cell contained unlabelled urea (0,01 M). All solutions were made isotonic by adding sodium chloride.

5.10.3 RESULTS

See table 5-10 and figure 5-12 for the relevant data on the permeation of ℓ-leucine and the co-permeation of ℓ-leucine and urea.

5.10.4 DISCUSSION AND CONCLUSIONS

The permeation of ℓ-leucine alone shows an exponential increase up to approximately 54 hours after which a plateau is seen with a permeability coefficient of approximately 0,009 cm/hr.

When the permeation of ℓ-leucine in the presence of urea is measured, the same general pattern is observed but the permeability coefficient is higher than without urea at the plateau phase. The permeability coefficient at the plateau phase is approximately 0,015 cm/hr. This means that urea has an enhancing influence on the permeability of ℓ-leucine, which might mean a reduced uptake of ℓ-leucine by the cells in the presence of urea.

The permeation of urea is significantly reduced in the presence of ℓ-leucine. The permeability coefficients obtained at 100 hours

Table 5-10 The permeability coefficients of ℓ-leucine for full thickness hairless mouse skin with and without simultaneous permeation of a 0,01 M solution of urea over a period of 100 hours

Time (hours)	Permeability coefficient x 10^3 cm/hr			
	ℓ-leucine		urea	
	alone [1]	with urea [1]	alone [2]	with ℓ-leucine [1]
5	0,2	0,2		0,3
11			0,3 (0,2)	
21			1,4 (1,4)	
23	1,1	1,5		1,8
25			6,9 (5,1)	
30	2,5	3,6		4,8
43			24 (10)	
51			87 (42)	
54	6,5	12		17
74			116 (60)	
77	9,2	26		36
96			200 (91)	
100	8,2	17		25

[1] The mean of two determinations.

[2] Standard deviation in brackets. Four detemination were made in each case.

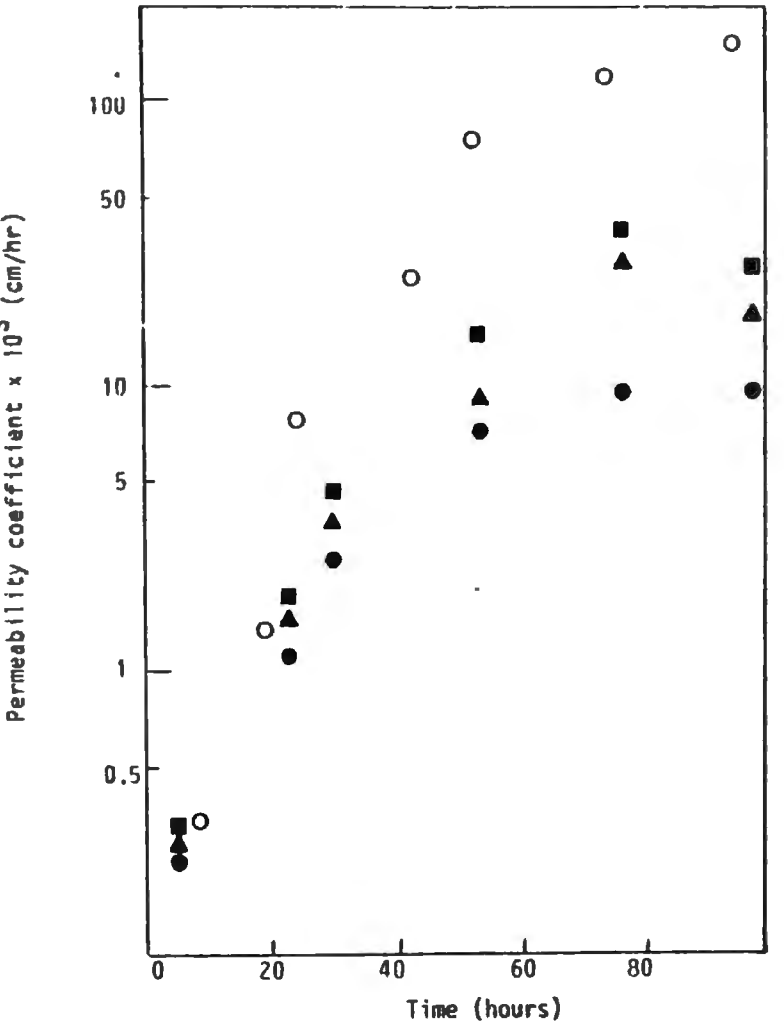

Figure 5-12 The permeability coefficients of ℓ-leucine alone -●- , ℓ-leucine in the presense of urea -▲- and urea in the presense of ℓ-leucine -■- and urea alone -○- as a function of time.

when urea permeates alone and when ℓ-leucine is co-permeated are respectively 0,1 cm/hr and 0,03 cm/hr.

The general conclusion is that ℓ-leucine retards the permeation of urea and that urea enhances the permeation of ℓ-leucine. The effect of ℓ-leucine on urea is more pronounced than the effect of urea on the permeation of ℓ-leucine. ℓ-Leucine thus plays an important role in retarding the deteriorating effect of urea or the hydration effect on the permeability barrier.

Only viable cells will consume or utilise ℓ-leucine. Since ℓ-leucine has an affect on the permeation of urea, it is an indication that the viable epidermis may play an important role as a permeability barrier to the permeation of urea and that the viable cell barrier may progressively deteriorate during experiments with a consequent gradual increase in permeability.

5.11 SUMMARY AND CONCLUSIONS ON THE INCREASING PERMEABILITY PHENOMENON

The effect of several variables on the permeation of urea through hairless mouse skin has been studied in order to determine the causes of the increasing permeability phenomenon found in the preliminary studies.

It was found that the permeation of urea increased for a period of approximately 100 hours after which a steady-state permeation pattern was observed for approximately 25 hours. A mass balance between the donor and receiver sides of the membrane was reached within a much shorter time - due to the very low permeability of urea in the first few hours of the experiment and the experimental variation this period can only be estimated as between 1 and 4 hours. Thus a very short "lag time" and mass balance even during the increasing permeability phase is seen.

Hydration was thought to be one of the possible causes of the increasing permeability phenomenon. In two experiments, one where the skin was hydrated for different time intervals before starting a permeation experiment and the other where a sequential run procedure was utilised, it was found that hydration or the effect of the bathing medium on the skin was the major cause for the increasing permeation. Urea was found not to affect its own permeation in a concentration of 0,01 M.

The question arose whether urea would effect its own permeation in higher concentrations than 0,01 M. After permeation studies with 0,27 M and 1,67 M it could be concluded that there was no significant difference between the permeation of urea in these concentrations that differed by two logarithmic orders. This

result confirmed that the permeating urea was not the cause of the increasing permeability.

It seemed as if the only alternative cause for the increasing permeability would be found in the isotonic bathing media. Two other isotonic media, MOPS and TRIS were used with a 0,27 M solution of urea and the results were compared to that obtained with the normal saline bathing media. When the experimental variation was taken into account, the permeation of urea in the presence of the MOPS and TRIS buffers followed the same pattern of increasing permeation as in the presence of normal saline.

Although it was found that contact with the isotonic bathing media was the primary cause of the increasing permeation, none of the above mentioned studies indicated explicitely a hydration or other mechanistic effect. It was therefore decided to compare the permeation of urea with that of other hydrophilic compounds for which a increasing permeability was not observed previously. In studies with water, methanol and ethanol (Treherne, 1956:171; Dürrheim et al., 1980:781) the increase in permeability as a function of time over such a prolonged period was not observed and the steady state phase was attained within one hour. If the difference between these compounds and urea are assosiated in some way with the heterogenous nature of the stratum corneum implying different permeation mechanisms for example for urea and water, one would expect that in a dual label experiment where ^{14}C-urea and ^{3}H-water are permeated simultaneously that urea will show the increasing permeability pattern while the permeability of water will not change as a function of time. This hypothesis was tested by conducting simultaneous runs of ^{14}C-urea, ^{3}H-water and ^{14}C-methanol. When the permeation patterns over the first six hours of these experiments were compared, it was clear that

urea, methanol and water exhibit the same increasing permeability pattern. Therefore it was concluded that no significant difference in the mechanism of and the barrier to the permeation of urea, methanol and water existed.

Although the stratum corneum is generally accepted as the main barrier to the penetration of substances through skin, it is also well known that hydration of the stratum corneum leads to an increase in permeability - although not to the extent seen in this study. When the stratum corneum becomes so much hydrated that it offers no significant barrier to the permeation of very hydrophilic compounds, the remainder of the epidermis might become the major barrier to the permeation of these compounds. From the results when urea permeated stripped skin it was unexpectedly found that the permeation through full thickness skin increased much faster than permeation through 10 times stripped skin. The only conclusion that seemed viable was that although stripping is incomplete it may remove the most heavily bacteria contaminated layers which reduced microbial attack on the permeation barrier and thus slowed down any detriorating effect the microorganisms might have had on the permeation barrier.

The remainder of the epidermis contains viable cells and if the permeability barrier depends upon the maintenance of normal cell function, the reduced uptake of an essential amino acid like L-leucine should be an indication of the change or deterioration of the permeability barrier during prolonged contact with the bathing medium. ^{3}H-L-Leucine and ^{14}C-urea were permeated simultaneously and it was found that the permeation of urea was significantly reduced in the presence of L-leucine. This may be an indication that the viable epidermis play an important role as a permeability barrier to the permeation of urea.

Although the cause of the increasing permeation of urea could not be explicitly identified, a base for the solving of this problem has been developed and several important observations on the permeation of urea and the behaviour of hairless mouse skin in an *in vitro* diffusion system have been made of which the following should be mentioned:

- urea did not affect its own permeability or that of water. The increasing permeability could therefore not be ascribed to the permeating urea;

- urea, methanol and water exhibited the same pattern of increasing permeability contrary to what had been expected from other studies with methanol and water. The mechanism of permeation seemed to be the same for these compounds and it seemed as if it was not the permeating substance that was responsible for the increasing permeability but another factor, common to all the experiments;

- it seems therefore to be safe to conclude that the increasing permeability phenomenon was caused by the effect of the bathing medium on the skin. This effect may however include various factors such as hydration, physical deterioration and provision of a growth medium for microbial proliferation - factors that should be studied more intensively in future studies.

CHAPTER 6 SUMMARY AND FINAL CONCLUSIONS

The following contributions towards the systematic characterization of the percutaneous absorption of drugs through the skin have been made in this study:

An existing data base on the relationship of ether-water partition coefficients and permeability coefficients was lacking data on the percutaneous absorption of hydrophilic compounds through hairless mouse skin *in vitro*. The permeability coefficients obtained in this study and their correlation with ether-water partition coefficients showed some errors of interpretation and approach in previous work on the percutaneous absorption of urea, thiourea, glycerol and glucose.

The data obtained in this study could be integrated with already existing data which confirmed the incompatibility of the hypothesis of a simple lipid membrane mechanism with the behaviour of skin. For solutes with a span in ether-water partition coefficients of approximately ten logarithmic orders, only four orders of magnitude change in permeability coefficients were noted.

The general significance of this result is that mathematical models of the skin barrier must allow for complex, concurrent channelling of permeant through lipid and hydrated phases, which means that the arrangement of phases must be such that the rate control can switch from lipid to aqueous pathways at several places on the polarity scale.

Since large molecules such as steroids appear to have a different

polarity dependency than small molecular weight permeants, it confirms the idea that access to microscopic phases within the stratum corneum may be controlled by size.

The systematic investigation of the increasing permeability of urea through full thickness hairless mouse skin in vitro led to the following conclusions:

- a steady state was reached after approximately 100 hours of increasing permeation;
- urea did not affect its own permeation between concentrations of 0,01 M and 1,67 M and it did not affect the permeation of water. The increasing permeation could therefore not be ascribed to the permeating urea;
- the effect of the bathing medium on the skin was responsible for the increasing permeability phenomenon. This effect did however not depend on the specific substance that was used to render the solution isotonic, since no significant difference between the permeation of urea in the presence of normal saline, MOPS or TRIS was found. This effect of the bathing medium may include several factors such as hydration, physical alteration of the skin and provision of a growth medium for micro-organisms. From the results of this study no specific mechanism for the increasing permeability could be indicated;
- the barrier of ten times stripped skin was less susceptible to deterioration after 40 hours of being in the diffusion medium than full thickness hairless mouse skin, probably because the stripping removed the most heavily bacteria contaminated layers of the skin and prevented part of the possible proliferation of micro-organisms in the diffusion medium;

- the co-permeation of urea and *l*-leucine seemed to suggest that the viable epidermis may be a more significant barrier to the permeation of hydrophilic compounds than was initially been anticipated.

It is tempting to ascribe the increasing permeation of urea and the other hydrophilic compounds to the extensive hydration of the skin in the specific in vitro system used. One should, however, be careful not to generalize the term "hydration" so far that it becomes a synonym for the possible combination of factors responsible for the deterioration of the permeation barrier.

The increasing permeation phenomenon may possibly be ascribed in part to the binding and penetration of water within the stratum corneum ultrastructure, which may lead to the formation of larger hydrophilic diffusion channels which may promote the permeation of hydrophilic substances as a function of time.

But there may be a myriad of other factors which may also play a role in changing the barrier - factors which cannot be disregarded in favour of hydration just because they have not yet been studied.

Factors that should be studied in the continuation of this systematic search for the cause of the increasing permeability phenomenon should be the physical deterioration of the barrier in the skin as a result of:

- the extensive contact with the diffusion medium. Components of the barrier phase may be dissolved or altered by the contact with the stirred medium on both sides of the skin; or

microbial attack of the skin structure, which may most likely occur under such hydrated conditions where sterile procedures are not followed. The microbial barrier of the skin is based on the dry, lipophilic and slightly acidic surface of the stratum corneum *in vivo*, factors which are all nullified in an *in vitro* system where the skin is mounted between aqueous solutions of the permeating substance.

The significance of this study may thus be summarized as:

1. it showed errors of interpretation in previous work on the percutaneous absorption of hydrophilic compounds;

2. it confirmed the incompatability of a simple lipid membrane mechanism with the behaviour of skin;

3. it formed a basis for the systematic solution of the mechanism of increasing permeability as a function of time phenomenon encountered with the studied hydrophilic compounds.

ABSTRACT

INTRODUCTION

The objective of this study was initially to

- contribute to a current data base in order to obtain a set of data on the ether-water partition coefficients and permeability coefficients of a variety of compounds ranging from very hydrophobic to very hydrophilic in order to assess
  - the relationship between hydrophobicity and percutaneous absorption; and
  - the validity of the view that the skin can be diffusionally characterized as a simple lipoidal barrier.

Data on the ether-water partition coefficients and the percutaneous absorption of a series of alkanols (Dürrheim, 1980:781) and hydrocortisone and its 21-n-alkyl esters (Smith, 1982:100) derived from in vitro work on hairless mouse skin already existed. These two series of compounds display relative little hydrophilicity. Therefore data on the percutaneous absorption of more hydrophilic compounds would have complemented the existing data very well.

The work of Treherne (1956:171) on the relationship between the ether-water partition coefficient and percutaneous absorption of a few polar nonelectrolytes has been much cited in the literature and was taken as a point of departure for this study. Since Treherne (1956:171) used other techniques, diffusion cells and rabbit skin, his data did not fit into the existing data base, which was

obtained using modern techniques, other cells and hairless mouse skin. Therefore percutaneous absorption studies of the selected hydrophilic compounds through hairless mouse skin had to be done in order to attain the goal of the study.

In the course of the permeability studies, a very slow but gradually accelerating permeation of the hydrophylic compounds was noted, a phenomenon that became very noticeable when the permeation profile was observed over longer periods e.g. 100 hours instead of the conventional 6-12 hours. The initial objective had to be expanded to search for the cause of the increasing permeability of these compounds. With urea reproduceable results were obtained in the preliminary study and therefore urea was chosen for this study.

METHODS

The percutaneous absorption studies of urea, thiourea, glycerol and glucose were executed by means of a previously described *in vitro* method (Dürrheim *et al.*, 1980:781). The method included the use of a diffusion cell system where the skin was clamped between two diffusion cells. The donor cell contained a relatively high concentration of the permeant while the receiver cell contained a just measurable very low concentration of the permeant during the full time span of the experiment. A fairly constant concentration gradient could therefore be maintained over the membrane and Fick's law could be applied to results obtained in

that manner. In the extensive study on the permeability beha= viour of urea under different conditions, the same diffusion cell system was used.

The ether-water partition coefficients of the hydrophylic com= pounds were determined by a simple radiotracer procedure. The radio-labelled compound was equilibrated between the ether and water phases and the samples taken from the phases were analysed by means of scintillation counting.

The ether-water partition coefficients of hydrocortisone and its 21-n-alkyl esters were determined by means of a HPLC method as previously described by Smith (1982:100). These coefficients could not be determined with scintillation counting since all the compounds were not available in radio-labelled form. The HPLC method had the advantage that the stability of the compound could be ascertained simultaneously.

RESULTS

The permeability coefficients of the hydrophilic compounds (gluco= se, urea, glycerol and thiourea) varied only from 1×10^{-4} to $1{,}4 \times 10^{-4}$ cm/hr while their ether water partition coefficients varied from 9×10^{-6} to $7{,}2 \times 10^{-3}$. There is no correlation be= tween the partition coefficients and permeability coefficients for these compounds, which might indicate that permeation of hydrophylic compounds with ether-water partition coefficients not substantially higher than that of water does not depend on the hydrophobicity of the compound. The mechanism of permeation

of such hydrophilic compounds through hairless mouse skin might therefore be mainly through the water-filled intercellular chan= nels in the stratum corneum.

The systematic investigation of the increasing permeability of urea through full thickness hairless mouse skin in vitro led to the following results:

- A rapid increase in permeation persisted until approximately 100 hours, after which the rate stayed constant for at least 24 hours;
- the bathing medium had a deteriorating effect on the permeation barrier;
- between concentrations of 0,01 M and 1,67 M urea did not affect its own permeation significantly;
- urea did not affect the permeation of water or methanol significantly;
- the barrier of ten times stripped skin is les suscep= tible to deterioration after 40 hours of being in the diffusion medium than full thickness hairless mouse skin; and
- the co-permeation of urea and L-leucine seemed to sug= gest that the viable epidermis may be a more significant barrier to the permeation of hydrophylic compounds than had initially been anticipated.

It seems as if there is a lower limit to permeability coefficients for hairless mouse skin. This agrees with the suggestion made by Scheuplein and Blank (1973:286) that there is a finite lower limit of approximately 10^{-6} cm/hr for human skin permeability coefficients. There seems to be an upper limit at approximately 0,5 cm/hr, which corresponds roughly to the permeability coef= ficients found for different compounds throuqh the dermis only of hairless mouse skin (Dürrheim, 1977:134; Smith, 1982:144).

That means that different diffusional pathways exist for compounds of different polarities and that lipophilicity is not the only determinant of diffusivity and rate of permeation. Consequently it can be concluded that the skin does not act as a simple lipoi= dal barrier with regard to compounds ranging much in hydrophobi= city.

It is tempting to ascribe the increasing permeation of urea and the other hydrophylic compounds to the extensive hydration of the skin in the specific in vitro system used. One should, however, be careful not to generalize the term "hydration" so far that it becomes a synonym for the possible combination of factors responsible for the deterioration of the permeation barrier.

The increasing permeation phenomenon may thus be ascribed to the binding and penetration of water within the stratum corneum ultra= structure, which may lead to the formation of larger hydrophilic diffusion channels and may promote the permeation of hydrophilic substances as a function of time.

But there may be a myriad of other factors which may also play a role in the deterioration of the barrier - factors which cannot be disregarded in favour of hydration just because they have not yet been studied.

Factors that should be studied in the continuation of this systematic search for the cause of the increasing permeability phenomenon should be the physical deterioration of the barrier in the skin as a result of:

- the extensive contact with the diffusion medium. Components of the barrier phase may be dissolved or altered by the contact with the stirred medium on both sides of the skin; or

- microbial attack of the skin structure, which may most likely occur under such hydrated conditions where sterile procedures are not followed. The microbial barrier of the skin is based on the dry, lipophilic and slightly acidic surface of the stratum corneum in vivo, factors which are all nullified in an in vitro system where the skin is mounted between aqueous solutions of the permeating substance.

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UITTREKSEL

INLEIDING

Die doelstelling van hierdie ondersoek was aanvanklik om

- 'n bydrae te maak tot 'n databank sodat 'n stel gegewens oor die eter-water verdelingskoëffisiënte en permeabiliteitskoëffisiënte van 'n verskeidenheid verbindings, wat van baie hidrofoob tot baie hidrofiel varieer, verkry kon word om sodoende
  - die verband tussen hidrofobisiteit en perkutaniese absorpsie en
  - die geldigheid van die siening dat die vel vir doeleindes van diffusie-ondersoeke as 'n eenvoudige lipiede weerstand gesien behoort te word, te bepaal.

Data oor die eter-water verdelingskoëffisiënte en die perkutaniese absorpsie van 'n reeks alkohole (Dürrheim et al., 1980:781) en hidrokortisoon en hidrokortisoon-21-n-alkielesters (Smith, 1982: 100) soos verkry deur in vitro metodes en haarlose muisvel was reeds beskikbaar. Hierdie twee reekse verbindings vertoon relatief min hidrofiliteit en die databank sou dus goed aangevul kon word met data oor meer hidrofiele verbindings.

Die bydrae van Treherne (1956:171) oor die verband tussen die eter-water verdelingskoëffisiënte en perkutaniese absorpsie van 'n paar polêre verbindings is baie aangehaal in die literatuur en is as vertrekpunt vir hierdie ondersoek geneem. Treherne (1956: 171) het egter ander tipe diffusieselle, ander tegnieke en konynvel gebruik. Sy data sou dus nie in die bestaande databank inpas

nie omdat die data met behulp van moderne tegnieke, ander tipe diffusieselle en haarlose muisvel verkry is. Perkutaniese absorpsiestudies van die gekose hidrofiele verbindings deur haarlose muisvel moes dus gedoen word om die doel van die ondersoek te bereik.

In die loop van die permeabiliteitsondersoek, is 'n stadige maar geleidelik versnellende oordrag van die hidrofiele verbindings opgemerk. 'n Verskynsel wat veral baie opvallend was wanneer die oordragpatroon oor langer tye, byvoorbeeld 100 ure in plaas van die konvensionele 6 tot 12 ure beskou is. Die aanvanklike doelstelling moes toe uitgebrei word sodat die oorsake van die toenemende oordragtempo van hierdie verbindings eers sistematies ondersoek kon word. Ureum het goeie herhaalbare resultate in die voorondersoek gelewer en is gekies om die ondersoek mee te doen.

METODES

Die perkutaniese absorpsiestudies van ureum, tioureum, gliserol en glukose is met behulp van 'n in vitro metode soos voorheen beskryf (Dürrheim et al., 1980:781), uitgevoer. Die metode het die gebruik van 'n diffusieselsisteem, waar die vel tussen twee diffusieselle vasgeklem word, ingesluit. Die skenkersel het 'n relatief hoë konsentrasie van die oordragstof bevat terwyl die ontvangersel 'n net meetbare baie lae konsentrasie van die oordragstof gedurende die volle duur van die eksperimente bevat het. 'n Konstante konsentrasiegradiënt oor die membraan kon dus gehandhaaf word en Fick se wet kon op die resultate toegepas word. In die uitgebreide ondersoek oor die permeabiliteit van ureum onder verskillende toestande, is dieselfde diffusieselsisteem gebruik.

Die eter-water verdelingskoëffisiënte van die hidrofiele verbindings is met behulp van 'n eenvoudige radiospeurmetode bepaal. Die radio-aktief gemerkte verbindings is toegelaat om 'n ewewig tussen die eter en water fases te bereik voordat monsters uit elke fase geneem is. Die monsters is met behulp van sintillasietelling geanaliseer.

Die eter-water verdelingskoëffisiënte van hidrokortisoon en die hidrokortisoon-21-n-alkielesters is met behulp van 'n hoëdrukvloeistofchromatografiese metode, soos deur Smith (1982:100) beskryf, bepaal. Hierdie verdelingskoëffisiënte kon nie met behulp van sintillasietelling bepaal word nie omdat al die verbindings nie in 'n radio-isotoopvorm beskikbaar was nie. Die hoëdrukvloeistofchromatografiese metode het ook die voordeel dat die stabiliteit van die verbinding terselfdertyd bepaal word.

RESULTATE

Die permeabiliteitskoëffisiënte van die hidrofiele verbindings (glukose, ureum, gliserol en tioureum) het van 1×10^{-4} tot $1{,}4 \times 10^{-4}$ cm/hr gewissel terwyl hul eter-water verdelingskoëffisiënte van 9×10^{-6} tot $7{,}2 \times 10^{-3}$ gewissel het. Daar kon geen korrelasie tussen die verdelingskoëffisiënte en die permeabiliteitskoëffisiënte van hierdie verbindings gevind word nie. Dit mag 'n aanduiding wees dat oordrag van hidrofiele verbindings, met eter-water verdelingskoëffisiënte van veel laer as 1, nie van die hidrofiliteit van die verbinding afhanklik is nie. Die meganisme van oordrag van sulke hidrofiele verbindings deur haarlose muisvel geskied dus moontlik deur die watergevulde intersellulêre ruimtes in die stratum corneum.

Die sistematiese ondersoek na die toenemende oordragtempo van ureum deur volle dikte haarlose muisvel in vitro het die volgende resultate opgelewer:

- 'n vinnige toename in oordrag hou vir ongeveer 100 ure aan, waarna die die tempo vir ten minste 24 uur konstant bly;
- die diffusiemedium is vir die veranderinge in die oordragweerstand verantwoordelik;
- tussen konsentrasies van 0,01 M en 1,67 M het ureum nie 'n noemenswaardige effek op sy eie oordrag nie;
- ureum beinvloed nie die vordrag van water en metanol noemenswaardig nie;
- die weerstand van tienmaal gestroopte vel is hoër na 40 ure in die diffusiemedium as dié van volle dikte haarlose muisvel;
- die gelyktydige oordrag van ureum en ℓ-leucine het die oordragtempo van ureum sodanig verlaag dat dit moontlik 'n aanduiding kan wees dat die res van die epidermis 'n belangriker weerstand by die oordrag van hidrofiele verbindings is as wat aanvanklik aangeneem is.

GEVOLGTREKKINGS EN AANBEVELINGS

Dit lyk asof daar 'n minimum permeabiliteitskoëffisiënt vir die oordrag deur haarlose muisvel is. Dié minimum waarde stem ooreen met die voorstel van Scheuplein en Blank (1973:286) dat 'n minimum waarde van 10^{-6} cm/hr ten opsigte van die permeabiliteitskoëffisiënte vir menslike vel bestaan. Dit lyk asof die maksimum waarde ongeveer 0,5 cm/hr is, wat min of meer ooreenstem met die

permeabiliteitskoëffisiënte wat by die oordrag van verskillende verbindings deur die dermis van haarlose muisvel gevind is (DÜrrheim, 1977:134; Smith, 1982:144). Dit dui daarop dat daar verskillende diffusieweë vir verbindings van verskillende polariteite bestaan en dat lipofiliteit nie die enigste bepalende faktor vir diffusiwiteit en tempo van oordrag is nie. Die gevolgtrekking kan dus gemaak word dat die vel nie soos 'n eenvoudige lipiede weerstand met betrekking tot verbindings oor 'n breë vlak van lipofiliteit optree nie.

Die versoeking is groot om die toenemende oordragtempo van ureum en die ander hidrofiele verbindings aan die uiterste hidrasie wat die vel in die betrokke in vitro sisteem ondergaan, toe te skryf. 'n Mens sal egter versigtig moet wees om nie die term "hidrasie" só te veralgemeen dat dit as sinoniem vir die moontlike kombinasie van faktore, verantwoordelik vir die verandering in die oordragweerstand, beskou word nie.

Die verskynsel van toenemende oordragtempo mag moontlik aan die binding en binnedringing van water in die ultrastruktuur van die stratum corneum in, toegeskryf word. Dit mag lei tot die vorming van groter hidrofiele diffusieweë wat die oordragtempo van hidrofiele verbindings kan bevorder.

Daar mag egter 'n magdom van ander faktore wees wat ook 'n rol in die verandering van die diffusieweerstand speel - faktore wat nie ter wille van hidrasie oor die hoof gesien mag word net omdat dit nog nie bestudeer is nie.

Faktore wat in die voortsetting van hierdie sistematiese ondersoek na die oorsaak van die verskynsel van toenemende oordrag-

tempo aandag behoort te geniet, is die fisiese agteruitgang van die weerstand in die vel as gevolg van:

- die kontak met die diffusiemedium. Komponente van die weerstandsfase kan oplos of verander word deur die kontak met die bewegende medium aan beide kante van die membraan; of

- mikrobiese afbraak van die velstruktuur. Iets wat baie waarskynlik is onder sulke gehidreerde toestande waar steriele prosedures nie gevolg word nie. Die mikrobiese weerstand van die vel is gebaseer op die droë, lipofiele en effens suur oppervlak van die stratum corneum in vivo - faktore wat almal uitgeskakel word in 'n in vitro sisteem waar die vel tussen twee waterige oplossings van die oordragstof gemonteer word.

Die belang van hierdie ondersoek kan dus soos volg opgesom word:

1. foute in die vertolking van resultate van vorige werk oor die perkutaniese absorpsie van hidrofiele verbindings is uitgewys;

2. die onverenigbaarheid van 'n eenvoudige lipiedmembraanmeganisme ten opsigte van perkutaniese absorpsie met die gedrag van die vel is bevestig; en

3. 'n basis vir die sistematiese ondersoek na die oplossing van die meganisme verantwoordelik vir die verskynsel van toenemende oordragtempo wat by die gekose hidrofiele verbindings opgemerk is, is daargestel.

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