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Methods of measuring, and factors affecting, percutaneous absorption

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Synopsis—Tests for PERCUTANEOUS ABSORPTION are needed principally for substances that are toxic or biologically active or which accumulate in body tissues. The most sensitive SPECIES for percutaneous absorption tests are the rabbit and the guinea-pig. The technique most widely applicable is the measurement by ANALYTICAL or ISOTOPE techniques of the rate of DISAPPEARANCE of a test substance from the site of its topical application. Urine examination or measurement of blood levels may be useful in certain circumstances. IN VITRO measurements are often useful as a guide to the planning of IN VIVO tests.

The principal barrier against percutaneous absorption is the STRATUM CORNEUM. Water and water-soluble substances traverse this layer with difficulty while LIPID-SOLUBLE substances do so with less difficulty. Substances that are soluble in both water and lipids (*amphipathic*) penetrate readily. Vehicles may retard or enhance percutaneous absorption in relation to their water-lipid solubility. They may also enhance absorption by increasing the permeability of the stratum corneum. In the case of OINTMENTS, partition of the test substances between vehicle and stratum corneum is often an important factor influencing absorption. Temperature and pH of the test preparations are additional factors that influence absorption.

INTRODUCTION

The penetration through the skin of compounds applied to its surface and the consequences of their systemic absorption has been of interest in the treatment of disease for a number of years (1). As far back as 1899 Mussey (2) demonstrated in the urine a number of drugs that had been applied to the unbroken skin. Two years later Reilly (3) reported that

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therapeutic effects could be obtained after cutaneous application of such substances as belladonna, mercury, pilocarpine and cod liver oil. This author also noted the presence of salicylic acid and phenol in the urine after their topical application to the skin.

Toxic effects, often serious and sometimes fatal, have been reported from substances applied percutaneously. Macht (4) reported deaths in experimental animals following the percutaneous absorption of a number of essential oils and Sannicandaro (5), and Gottlieb and Storey (6), reported deaths in humans from the absorption of salicylic acid and phenol through the intact skin. In recent years several examples of poisoning with organophosphorus compounds from percutaneous absorption have been reported both in humans following accidental contamination of the skin (7, 8), and in animals used in experimental studies (9). A number of other compounds have been reported to have caused toxicity in humans through percutaneous absorption. These include the chlorinated hydrocarbon insecticides, carbon tetrachloride, aromatic amines, dinitrophenol and hydrogen cyanide gas (10).

These observations have created an awareness that compounds present in many consumer products including toilet preparations, cosmetics and household materials, could produce untoward systemic effects by virtue of percutaneous absorption. Examples of this sort are the biologically active substances, intended for use in cosmetics, that are reported from time to time in the literature. One may recall here the mercaptans used in 'cold-hair wave processes' (11), steroid hormones intended to beautify the skin (12) and antibacterial agents (13). The recent reports indicating that hexachlorophene, added to a variety of toiletry preparations as an antibacterial agent, is absorbed percutaneously in amounts considered to be of toxicological hazard for babies (14) underlies the importance of keeping under review the risks that may arise from the inclusion of biologically active substances in cosmetic and toiletry preparations. Awareness of the possible toxic effects that may arise from these sources has led to the discovery that some cyclosiloxanes, originally developed for inclusion into cosmetic creams, are potent gonadotoxic and embryotoxic compounds (15). This was done during preliminary toxicological investigations on animals involving percutaneous absorption tests.

In order to assess potential toxicological hazard from this route it is important to have an understanding of the principles underlying the methods of testing and of the factors which influence percutaneous absorption. Both these fields have been extensively reviewed over the past two decades, but most reviewers tend to emphasize certain specialized aspects of the problem

(1, 4, 10, 16–37). The object of the present review is to deal with the principles of percutaneous absorption from a broader view point, bearing in mind the needs of those involved in the biological testing of these compounds. In attempting to delineate these principles we have taken into account lessons learnt from experimental results with a number of compounds of importance to the cosmetic chemist. These include alcohols, soaps, detergents and emollients. Although these are not absorbed from the site of topical application to any meaningful extent, they may produce profound local changes which affect the permeability of the skin to other, possibly toxic, compounds. In addition to the experience gained from this source, we have drawn upon the extensive experience of work done in the field of pharmaceuticals, pesticides and basic research, wherever appropriate.

THE CUTANEOUS BARRIER

Nature of the barrier

The passage of water and other substances into or out of the organism is limited by the skin which in mammals consists basically of an acellular layer of keratin and one or more layers of viable cells underneath. This 'barrier' function resides almost entirely in the stratum corneum (38, 39), a thin membranous layer (in the human 600 μm in the palms and soles, 10–15 μm in other parts) which is mechanically strong and is capable of resisting chemical attack (38). The importance of this layer in maintaining an adequate barrier was demonstrated by Blank (40, 41). He developed a method for stripping the stratum corneum in successive layers and showed that the rate of water loss, under standardized experimental conditions increased from 0.5 mg cm^{-2} h^{-1} to 8 mg cm^{-2} h^{-1} after complete stripping of the stratum corneum. Removal of the outer layers had little effect on the rate of water loss indicating that the main barrier occurs at the deeper layer which histologically is the stratum lucidum. The stratum lucidum is an equally effective barrier against the penetration of substances from the outside and its stripping considerably increases the penetration of topically applied substances. This was demonstrated by Blank, Griesemer and Gould (42) in their studies on the percutaneous absorption of the organophosphorus insecticide 'sarin' from the skin. The absorption of sarin after six strippings did not differ from that of controls, but after 12 strippings there was a hundred-fold increase. Damage to this layer also results in increased permeability to a variety of chemical agents (43–45).

The effective performance of this 'barrier function' appears to depend on the chemical composition of the stratum corneum. Chemical analysis has shown that this layer is rich in 'solid' matter which consists principally of keratin fibrils and lipid. These components are derived from the stratum spinosum, the layer of living cells immediately beneath the stratum lucidum (33, 46). The investigations of Matolsty, Downes and Sweeney (47) have shown that removal of the lipid or the protein of the stratum corneum results in an increased permeability to water indicating that both are essential to maintain intact the barrier property of this layer.

As one would expect, the thicker the stratum corneum, the more impermeable is the barrier. Marzulli (29), Blank and Scheuplein (33) and McCreesh (48) have shown that the rates of penetration of a wide variety of liquids applied to the epidermal surface *in vitro* were inversely related to the thickness of the epidermis, so that the soles and palms are much less permeable than the skin in other parts of the body. It would appear, however, that this rule does not apply *in vivo*. In a study of the comparative rates of absorption of parathion, malathion and carbaryl from a number of anatomic sites, the ^{14}C-labelled compounds were absorbed with equal facility from the palm or the hand or from the forearm. The abdomen and dorsum of the hand had twice the penetration of the forearm, whereas follicle-rich sites, including the scalp, angle of the jaw, the area behind the ear and the forehead, had a four-fold greater penetration (49), indicating that the skin appendages may constitute an important pathway for absorption.

The role of the appendages

The barrier formed by the stratum corneum is broken by the ducts of numerous glands and hair follicles, which, as indicated above, form an additional route by which substances applied to the skin surface might gain access to the circulation. In the human, the density of sweat ducts is 210 and 220 cm^{-2} in abdomen and forearm respectively (50), while that of the hair follicles is 40 and 100 in these same areas. This density is, however, variable—both between different anatomical areas and between species.

The presence of this additional route of percutaneous absorption has been verified by a number of experiments. Applying dyestuffs on the skin *in vivo* Rein (51) showed that the vicinity of follicles is stained more intensely than the rest of the epithelium. He also found that perifollicular staining does not occur around follicles which have been occluded by waxy plugs. These observations were supported by the work of MacKee, Sulzberger,

Hermann and Baer (52) who studied the penetration of heavy-metal compounds, sulphonamides and dyes through the skin of guinea-pigs and men, using histochemical techniques for the identification of these compounds. Further evidence has been provided from experiments using isotope-labelled compounds: for example, in studies of the passage of labelled parathion through the skin of a variety of species, including man, a high concentration of labelled material was found in the appendages (53) suggesting a preferential absorption via this route. In an appraisal of these autoradiographic results Fredriksson (54) suggested that they could equally well be regarded as an affinity of the test substance for certain structures. The same critical comment could be applied to observations on the localization of coloured compounds or on the histochemical demonstration of other compounds. Percutaneous passage by way of skin appendages was elegantly demonstrated by Van Kootan and Mali (55). Employing *in vitro* techniques, these authors mounted a freshly obtained piece of cadaver skin in a *Perspex* chamber and perfused one side of it with potassium ferrocyanide, and the other side with ammonium ferrisulphate. The solutions diffused through the epidermal barrier and formed a blue precipitate (Turnbull blue) at the site where they met. Under these conditions it was shown that a precipitate of Turnbull blue was formed at the epidermal barrier and within the skin appendages, especially sweat glands.

The contribution of sweat glands and of hair follicles to the percutaneous absorption of test substances has been investigated by a number of workers. Substances that are absorbed via the appendages must first pass through the orifices at the skin surface. According to Scheuplein (50) this process takes place by diffusion since 'hydrodynamic flow is excluded'. Taking into account the differences in the diffusion constants of the keratinized epidermis and the cells lining the ducts of the appendages, this author calculated that the route via the glands is important for a brief period immediately after the application of the test substance. During this period, absorption is greater through the appendages than through the matrix of the stratum corneum. Later a 'steady state' is established during which the bulk of diffusion appears to be no longer intra-appendageal but occurs through the matrix of the stratum corneum (50). In his studies, Scheuplein (50) considered only water and water-soluble substances and precluded the effect of solvents such as chloroform and methanol since these destroy the stratum corneum and as a consequence alter considerably the dynamics of absorption. In the case of lipid-soluble substances which do not affect the integrity of the stratum corneum to any appreciable extent, absorption via the pilosebaceous

apparatus may be considerable, due to its lipid-rich sebaceous secretion (50). The results obtained by Maibach *et al* (49) in their study of the percutaneous absorption of the lipid-soluble insecticides, parathion, malathion and carbaryl, mentioned in an earlier section, give considerable support to this hypothesis.

Further light on the relative rates of absorption via the skin appendages and the stratum corneum was provided by Wahlberg (22, 56). He used a solution of $^{22}NaCl$ and applied it to the shaved abdominal skin, rich in hair follicles and to the non-hairy skin behind the ear in guinea-pigs. From *in vivo* and *in vitro* experiments lasting several hours he showed that the percutaneous absorption from the abdominal skin was approximately 20% greater than that from the non-hairy skin and attributed this difference to the amount absorbed from the greater number of hair follicles in the abdominal skin. This conclusion was supported by a second experiment in which he used the same technique but substituted $HgCl_2$ for the sodium chloride. No difference in the absorption rates of Hg was observed between the hairy and non-hairy skin. This was due to the occlusion of the hair follicles and their glands by the protein precipitated by the mercury. These results suggest that even during the 'steady state', absorption via the hair follicles forms an appreciable fraction of the total amount absorbed. It would seem reasonable to assume that the sweat glands, if present, contribute an amount at least equal to that of hair follicles to percutaneous absorption.

While the hair follicle may complicate measurements of the amount absorbed, the hair keratin may interfere with measurements of the amount of the compound retained in the skin (57). In the design of experiments of this sort, it is important to remember that considerable regrowth of hair may occur, especially in young animals, within a few days of shaving.

Methods for testing

Efficiency of the cutaneous barrier

Of the procedures that have been employed for measuring the barrier properties of the skin, the one most frequently used is the water diffusion test, carried out under controlled humidity conditions *in vitro* (58, 59). A cylindrical aluminium cell containing water is separated from the atmosphere by a piece of skin which serves as a membrane. Water loss through skin after an appropriate equilibration time is expressed as mg cm^{-2} h^{-1}.

The permeability coefficient can be calculated if the water vapour gradient across the membrane is known. By appropriate experimental manipulations a comparison between the water loss through intact skin and through its component layers can be carried out. Studies of this sort have shown no change in the rate of water loss whether one uses whole skin, a preparation consisting of epidermis and stratum corneum or stratum corneum on its own.

Vinson (60) conducted a comparative *in vitro* study of the rate of diffusion of water through normal skin from neonatal and adult rat, from the adult guinea-pig and swine, and from male and female adult human subjects. The skin from neonatal rat was the least permeable exhibiting the lowest diffusion values (0.15 mg cm^{-2} h^{-1}). The diffusion values for adult rats and guinea pig skin and for specimens from the back or abdomen of adult swine were about four to five times that of the skin from neonatal rats. Values for human skin varied between these two extremes. Specimens from the female thigh gave diffusion values close to those of the neonatal rat. Others taken from the calf or from the abdomen in the male gave values close to those derived from adult swine The skin from the sole of the foot, taken from an adult female gave values of 2.1 mg cm^{-2} h^{-1}.

A simpler method for assessing the barrier properties of the stratum corneum is the measurement of its electrical conductivity. In the intact skin electrical conductivity is of the order of 1 μA V^{-1} and it is increased considerably after skin damage by abrasion or chemical action (60). Thus, immersion of guinea-pig skin *in vivo* at 70°C for 45 s, increased the conductivity from the control value of 0.04–0.9 μA V^{-1} to 2.2 μA V^{-1}. The increased rate of conductivity correlated with histological damage and with increased water diffusion over the range 0–10 μA V^{-1}. Application of dimethyl sulphoxide (DMSO) in concentrations of 20% or higher decrease considerably the electrical resistance of the skin (61). At such concentrations DMSO damages the keratin layer (62). A simple apparatus for electrical conductivity measurements has been described by Blank and Finesinger (63) and can be readily constructed.

In practice, measurement of the effectiveness of the cutaneous barrier does not form part of the routine tests for measurements of percutaneous absorption. The water diffusion test is too elaborate to be recommended for inclusion in tests of this sort. The electrical conductivity test on the other hand is simple and could be readily carried out. It could be useful as a screening procedure where enhancement of percutaneous absorption is suspected to be due to damage to the keratin layer or to an increase in its

water content. However, because of the limited knowledge of the factors influencing skin conductivity, this test cannot be recommended to replace the more orthodox methods.

Percutaneous absorption in vivo

'*Disappearance*' *technique or* '*analysis by difference*'

The techniques employed for the application of the test material on the skin surface and for measuring its percutaneous absorption vary considerably in detail but conform to a few general principles.

A measured volume of the compound is applied to the skin if the compound is a liquid in its natural state. *Parathion* and *Sarin* are examples of compounds applied to the skin in undiluted form in order to estimate their rates of absorption in experimental animals. If the test compound is a solid, a known amount is usually dissolved in a specified volume of a liquid vehicle and a measured volume of this solution is applied (19, 54, 64–66).

Occasionally it may be desired to deposit a very small quantity of test material in solid form on the skin. It is often more practicable to apply the material in a volatile solvent and to allow the solvent to evaporate quickly rather than to apply the material directly. Thus, Feldmann and Maibach (67) in their studies on percutaneous penetration of steroids in man applied the test steroid dissolved in acetone. Evaporation of the acetone was assisted by blowing a stream of air over it. It would appear that this procedure assists percutaneous absorption by increasing the local concentration (68). On occasions it may be essential to estimate the percutaneous absorption of a test substance contained in an ointment base. A weighed amount of the ointment, containing a known concentration of the test material, is usually rubbed on the skin in a defined manner and protected with a non-absorbable covering. Radio-active emission may be measured through this covering (69–71). An accurate ($\pm 10\%$) and simple method has been developed by Hadgraft, Barrett and Sarkany (72). It consists of producing a disc of the preparation on a small square of polyethylene by means of a thin tin sheet into which a circular hole has been cut. The tin sheet is placed over the polyethylene and a quantity of the test preparation is drawn across the hole by means of a spatula. The polyethylene square containing the disc of ointment is then removed and applied to the skin surface.

One of the first methods employed in the investigation of absorption through intact skin in living animals was devised by Hediger (73) and called

the method of 'analysis by difference'. This method involves the application to the skin of an accurately weighed amount of the penetrant and the estimation of how much remains in the deposit after different periods of time. In the original method, used extensively with little modification until recently, Hediger (73) used a bell-shaped glass vessel cemented to the skin. Samples were taken periodically from the skin enclosed within the vessel and removed for chemical analysis. This method often needed elaborate analytical procedures and was limited by the sensitivity of the analytical method employed (21, 74).

The advent of isotope labelling has considerably simplified the method of 'analysis by difference' since the amount remaining on the skin could be estimated relatively easily from its radio-active emission. These radio-active techniques have introduced difficulties of another sort, since the type of radio-active emission could have a considerable influence on the sensitivity and accuracy of the measurements. Gamma emission traverses readily the thickness of the corneum and epithelium so that compounds labelled with such isotopes do not offer any major difficulties in detection but the true passage of such isotopes through the cutaneous barrier may not always be readily assessed since the high penetrating properties of the gamma emission may give a positive result from regions beyond the cutis vera, e.g. subcutaneous tissue. On the other hand, β-emission does not possess such great penetrating properties so that failure to detect radio-active emission need not necessarily imply complete absorption: substances labelled with β-emitting isotopes may, for example, lodge in the skin appendages without detection (75–77). These complicating factors may affect materially the results, and it is essential to take these factors into account. For this purpose some workers excise the skin and subcutaneous tissue after completing a series of counts *in vivo* and measure separately the residual radio-activity in those two tissues (57, 76). The type of correction applied then depends on the type of radio-active emission. In the case of γ-radiation, any residual radio-activity in the subcutaneous tissue is subtracted from the readings obtained *in vivo* since the fraction of the compound responsible for this emission has passed through the skin. Residual activity in the subcutaneous tissue from β-emission need not be taken into account since it is unlikely that it would affect readings taken at the skin surface. On the other hand, residual β-emission in the skin itself needs to be taken into account since it represents radio-active material deep in the skin (usually pilosebaceous apparatus) which may have escaped detection (74).

A few selected examples may serve to illustrate the way in which per-

cutaneous application of isotope-labelled test substances and measurement of their absorption by the method of 'analysis by difference' is carried out in practice. Wahlberg (74) studied the absorption from guinea-pig skin of a number of metals labelled with γ-emitting isotopes. The animal was anaesthetized, the hair was clipped and a glass cylinder of an inner diameter 20 mm (exposure area 3.2 cm^2) and 104 mm high, was glued to the clipped area of skin. The glue was allowed to dry for 24 h and 1.0 ml of the respective isotope-labelled solution was put in the glass cylinder. This was immediately covered with a cover glass in order to prevent evaporation. The lower edge of the collimator was then placed in contact with the glass coverslip. Counting was commenced 15–30 s later and readings were taken over a period of 5 h. In order to estimate to what extent the amount of test material in the various layers of the stratum corneum, the epidermis or subcutaneous tissue influence the overall reading, the initial depot was carefully wiped off and the underlying skin was successively stripped in layers by 35 successive applications of adhesive tape. Results of a series of experiments indicated that the content of the radio-active label of the various skin elements varied greatly and it was not found possible to estimate the extent to which the 'disappearance curve' is influenced by amounts present in skin layers and subcutis at any given moment of time, so that the best that can be achieved is a measurement of the sum of radiation from the various layers.

The possible sources of error from this type of experiment were found to be injury of the skin by the adhesive used to secure the glass ring, absorption of the isotope to the glass and leakage from the depot. Error from these sources could be guarded against by suitable preliminary work and careful attention to detail.

In another type of experiment, the treated skin is excised after a predetermined period of time and the radio-activity is then measured. This method was employed by Parekh *et al* (57) in determining the percutaneous absorption of sodium pyridinethione (SPT). ^{35}S labelled SPT was applied in soap solution to the abdominal skin of rats 24 h after shaving. The application was spread over a fixed area by a *Teflon* rod, and was prevented from spreading further by the use of a polyethylene O-ring. After 15 s the treated area was wiped clean, the skin and subjacent muscle from this area were digested by an appropriate solubilizer, and the radio-activity of the digest estimated in a scintillation counter. The same method, with slight modifications, was used to determine skin absorption in monkeys. No cover was applied to the treated area presumably because of the short duration of the experiment.

Although 'disappearance measurements' are essential in order to obtain direct evidence of absorption of the test substance from the site of topical application, some idea of the rate of absorption may be obtained by measuring its rate of excretion in urine or faeces, from its deposition in internal organs, or by observing some known biological effect as a consequence of absorption. The list of compounds studied in this way in both animals and humans is an exhaustive one and only representative examples can be mentioned here.

The absorption of mercury from intact and abraded skin was investigated critically by Sorby and Plein (70) by the use of ^{203}Hg. These workers used ammoniated mercury labelled with ^{203}Hg applied to the skin in the form of an ointment, and, 24 h later, the kidneys were removed for the determination of mercury content since after absorption the metal accumulates chiefly in this organ (77). Estimation of the radio-active label in the urine has been found useful in other investigations especially in the investigation of the percutaneous absorption of steroids (67). The radio-active label, in this series of investigations, was found useful not only in determining the rate of absorption but in rendering unnecessay the elaborate analytical procedures required for identifying and measuring the metabolites. In other experiments, the radio-active label has been found useful in assisting the isolation and identification of the metabolites. For example, the presence of ^{35}S in sodium pyridinethione was found to be of considerable assistance in the identification of its metabolite, pyridine-N-oxide-2-sulphonic acid in the urine of rats after dermal application of the parent compound (78).

The pharmacological or other known biological effects of a particular compound can sometimes be used to investigate percutaneous absorption. Topical application of microgram quantities of steroids incorporated in a cream or ointment base produce local vasoconstriction which is visible as blanching. The degree and extent of blanching by topical corticosteroids was suggested as an indicator of percutaneous absorption and as a means of comparing absorption and efficacy in tests for new steroids (79, 80). Further experience indicated that this method of testing was neither accurate nor reproducible; the degree of blanching was subject to 'observer error' and was found to vary in the same individual at different times of day even though the same anatomical site was used. The surrounding vascular skin colour and degree of pigmentation were found to interfere considerably with the interpretation of results. Dissolving the steriod in ethyl alcohol did not appreciably improve the reproducibility of the vasoconstriction (81). Despite these limitations, this method gave a reasonably close approximation

between vasoconstrictor ability and clinical efficiency (82–84). The production of an area of anaesthesia by a topically applied substance could be used as a means of detecting percutaneous absorption, but it is even more subject to error than the vasoconstriction test (85).

Other pharmacological parameters have been found useful in determining percutaneous absorption and are still used occasionally in order to relate pharmacological action with rates of absorption measured by other tests. For example, changes in serum cholinesterase have been used to compare the toxicity of parathion and paraoxon after dermal application (54, 86, 87).

Other criteria which have been used occasionally are death of test animals (so-called cutaneous LD_{50}) from topically applied compounds or organ damage assessed histologically. Such an approach does not give an accurate measurement of percutaneous absorption but may be useful in order to obtain data on the dose levels at which certain compounds may produce systemic toxicity when applied topically. This approach was used by Wahlberg (74) in a comparative study of the systemic toxicity of mercuric chloride, cobaltous chloride and sodium chromate and is extensively used in determining the dermal toxicity of pesticides (88).

It is not always possible to compare the results of percutaneous absorption using an isotope-labelled compound with those obtained using other methods of measurement. In many instances the sensitivity of the analytical techniques employed is very much less than the radio-isotope techniques so that meaningful comparisons are difficult. Antibiotic assays using microbiological techniques are sensitive and accurate and Vickers (89) compared the percutaneous absorption of sodium fusidate and fusidic acid, using such techniques with the result of absorption obtained by standard ^{14}C-labelling techniques. Both by *in vivo* and *in vitro* methods, the results were found to be very close confirming the reliability of the radio-isotope techniques.

The measurement or demonstration of skin absorption using biological effects is limited to compounds having a high biological activity. The use of this technique for cosmetic ingredients is therefore limited but is important in the case of biologically active materials used in some permanent waving solutions or that will control bacteria on the skin, influence metabolism in such ways as to improve the texture or appearance of the skin, retard perspiration, or control dandruff.

Autoradiography

Autoradiography has been employed in the study of percutaneous

absorption *in vivo* (54, 91). This technique is useful in determining the presence of the compound in the various anatomical layers of the skin and in gaining some idea of the relative concentration. However, it is of limited value for quantitative investigations.

Enhancement of penetration

It is sometimes necessary to simulate 'conditions of use' where the skin surface may be exposed to injury and loss of its protective keratin expected. One method frequently employed is to remove keratin by the successive application and stripping of adhesive cellulose tape to the same cutaneous site. The number of consecutive strippings is usually about 25 (53, 92). The depth of epidermis removed by the adhesive is not uniform. Histological studies of tape removed after firm application revealed that in some areas the complete epidermal barrier is removed but in other areas only the merest trace of keratin (93). Other authors claim that a uniform separation of the keratin barrier is achieved by this method (29) so that the barrier is uniformly weakened. The skin barrier can be removed also by other methods. Recently Parekh *et al* (57) scarified the skin with a 'dull' razor to the point of localized bleeding in order to assess the absorption of sodium pyridinethione from damaged skin.

Percutaneous absorption 'in vitro' methods

In vitro measurements are found particularly useful in comparing the rates of diffusion of different compounds (74, 94, 95) and in obtaining some idea of the rate of the transepidermal passage of highly toxic substance prior to *in vivo* tests (42).

They also provide a means of obtaining a better understanding of the factors that influence percutaneous absorption *in vivo* (9, 96)

Both human and animal skin have been used for *in vitro* studies. Human skin is obtained at necropsy generally from the abdomen (42, 97) and from the abdomen, flanks or back in the case of animals (98, 99). Although the methods employed for the *in vitro* measurement of percutaneous absorption vary considerably in details, they follow a general pattern. The specimen of skin is trimmed to a suitable size and is mounted in a hollow chamber so that it divides the chamber into two compartments. The two surfaces of the skin are bathed in a suitable fluid. One of the fluids contains the test substance. Passage through the skin is then measured either by the 'disappearance' of the test substance from one chamber, by its appearance in the other or by both methods combined. Full details of the construction of

a chamber suitable for such experiments and of the techniques employed are found in other publications (42, 94–98, 100, 101).

The applicability of *in vitro* techniques is largely due to the non-cellular nature of the main epidermal barrier, the keratin layer, so that vital processes dependent on the integrity of epidermal cells are not involved: these cells lose their viability soon after death or removal from live animals.

Knowledge of the rate of transepidermal passage of chemicals is important in an assessment of the hazard of systemic toxicity from chemicals applied percutaneously. The *in vivo* method of 'analysis by difference' using a suitable radio-active technique is the most appropriate for assessing percutaneous absorption of cosmetic compounds. If such a study is first carried out in animals it may need to be supplemented by *in vitro* studies on excised human and animal skin in order to obtain some idea of the expected rate of penetration in humans. *In vitro* studies may also assist in studying factors which influence absorption of specific chemicals under defined conditions, such as temperature and pH.

Factors influencing percutaneous absorption

Physico-chemical factors

Temperature

In most experiments on percutaneous absorption the environmental temperature is kept at about 37°C. Under the ordinary conditions of life, however, the skin temperature fluctuates considerably, especially in the exposed parts. Fluctuations in skin temperature are known to influence percutaneous absorption. Whitehouse, Hancock and Haldane (102) in their study of the passage of water and gases through the human skin, demonstrated an increase in the rate of percutaneous absorption of oxygen on raising the environmental temperature of man. Brown and Scott (103) later showed an increase in the absorption of methyl salicylate due to an increase in the skin temperature. Fritsch and Stoughton (104) investigated the effect of temperature on the *in vitro* percutaneous absorption of ^{14}C acetylsalicylic acid on human skin. They found that at 40°C and 88% rh, the transepidermal passage of salicylate was about eight times times greater than it was at 10°C. An increased rate of percutaneous penetration of alcohols (C_2–C_8) over the range of temperature 5°–50°C was observed by Blank, Scheuplein and MacFarlane (105) using *in vitro*

technique. They determined the permeability constants of each of the seven alcohols and found that there was, on an average, a 10-fold increase in the permeability constant for each alcohol, as the temperature rose from 10 °C to 50°C. A five-fold increase in the rate of absorption of salicylic acid and carbinoxamine was also found *in vivo* when the skin temperature of the abdomen of guinea-pigs was raised from 20°C to 38°C (106). These effects of temperature demonstrated by *in vivo* and *in vitro* techniques are not due to skin circulatory effects of heat and cold.

State of ionization

Before the advent of radio-isotope labelling techniques it was generally held that electrolytes applied to the mammalian skin in aqueous solutions either do not penetrate at all, or if they do, they enter only in small amounts (21). In several experiments 'non-physiologic' cations, such as Li, Hg, Cs, Sr and Ba or of 'physiological' cations such as Na and Ca were applied to the skin of several species including man. No transepidermal passage could be detected by the analytical techniques employed (107–111). With the isotope tracer technique Loeffler and Thomas (112) were able to demonstrate the percutaneous absorption of aqueous radio-active strontium solutions ($^{89}SrCl_2$) through the shaved skin of rats. Johnston and Lee (113), applying an aqueous solution of $^{23}NaCl$ incorporated in an ointment base to the right arm of man, showed that radio-activity appeared in the left hand and in the urine. Others (96, 114, 115) have confirmed that electrolytes penetrate mammalian skin and in a recent experiment Wahlberg (56) showed that in the guinea-pig approximately 20% of the amount of $HgCl_2$ and NaCl applied to the skin was absorbed via the skin appendages, the remaining 80% passed via the stratum corneum. It is of some interest that the rate of diffusion of water estimated by Scheuplein (39) on human epidermis *in vitro* is of the same order as that of the electrolytes employed by Wahlberg (56) on guinea-pig skin. This indicates that the rate of penetration of these electrolytes is not appreciably different from that of water. It would seem reasonable to expect other electrolytes to penetrate the skin at similar rates. Skog and Wahlberg (114) applied the chlorides of Co, Zn, Cd and Hg, sodium chromate and silver nitrate in equimolar concentrations (0.002 M) to the skin of guinea-pigs. With the exception of $HgCl_2$ these salts are present in an ionized form and their rate of absorption on a molar basis was of the same order as that of water. Some variation in the percutaneous absorption, expressed as disappearance constant was, however, found. The mercury compounds were absorbed twice as rapidly as those of Co,

Zn and Hg, while cadmium occupied an intermediate position. Non-electrolytes are influenced by other factors which will be dealt with in a subsequent section.

pH

The pH of the solution influences percutaneous absorption principally by determining the state of ionization of a particular compound. Samitz *et al* (97) found that the absorption of chromium from chloride, sulphate or nitrate solutions *in vitro* was less at a pH of 7 than at pH 5 or pH 9. Later studies *in vivo* by Arita *et al* (106) confirmed that altering the pH of the skin to either side of neutrality increased absorption. Thus, absorption of salicylic acid was less at a pH 5 or higher than that at more acidic pH values while with carbinoxamine, absorption was lowest at pH 7 and increased as the pH became more alkaline. In both these investigations the authors attribute the low absorption at neutral pH to a higher degree of ionization at this pH than at more acid or alkaline pH values. If the solution is strongly acid or alkaline an irreversible destructive effect on the keratin occurs which will render the skin more permeable (1).

Water/lipid solubility

A much wider variation in the rate of absorption has been demonstrated in studies of some non-electrolytes. Skin permeability to a homologue series of normal primary alcohols C_1–C_8 applied in dilute aqueous solutions were studied by Blank (46). He found that methanol and ethanol (0.1–0.4 M) penetrated the epidermal barrier *in vitro* to the same extent as water. The rate of penetration of propanol was greater than that of water by a factor of 1.4. The higher alcohols in this series penetrated the skin much more rapidly; octanol, the alcohol with the highest molecular weight in the series, passed through the barrier 52 times as fast as water. The lipid solubility of these alcohols also increased with increasing molecular weight, and according to Scheuplein (39) the increased rate of absorption is accounted for by the increased lipid solubility. At higher concentrations, the rate of penetration of the alcohols is greatly increased, and does not follow the pattern of absorption from weak solutions. According to Blank and Scheuplein (116) this may be due to one or other of two reasons: at high concentrations, the alcohol content of the stratum corneum increases and this 'acts as an added pathway'. Secondly, high concentrations may damage the stratum corneum impairing its 'barrier' properties.

An even greater difference in percutaneous absorption was shown by the series of non-electrolytes studied by Treherne (117). This author determined the permeability of skin to ethyliodide, methanol, ethanol, thiourea glycerol, urea and glucose and found that it decreased in this order. He also found that the permeability of ethyliodide was a hundred times that of glucose. Since the rates of transepidermal diffusion agreed with the calculated rates of diffusion from water into lipid, the author concluded that the principal factor determining the rate of diffusion was the degree of lipid solubility. As in the case of the alcohols, molecular volume appears to have little effect, for example, the molar volume (expressed in ml) of ethyliodide is 25.23 while that of urea is 13.67.

The influence of lipid solubility on percutaneous absorption was studied by Wurster and Kramer (26) using the three salicylate esters: ethyl-, methyl-, and ethylene-glycol esters. They showed that under conditions of normal hydration of the stratum corneum, the *in vivo* absorption for ethyl- and ethylene-glycol salicylate was 1.5 $\text{M} \times 10^5$ 100 cm^{-1} h^{-2}. Absorption was about twice this rate for methyl-salicylate which had a greater water/lipid partition coefficient than the other two esters. Since the compounds studied by Blank (46), Treherne (117) and Wurster and Kramer (26) possessed different degrees of water solubility in addition to their lipid solubility, it would appear that the water/lipid partition coefficient, rather than lipid solubility as such, is the important factor. The work carried out by Clendenning and Stoughton (118) and Marzulli, Callahan and Brown (95) supports this view. Clendenning *et al* (118) studied carefully the relation between the percutaneous absorption and water/lipid partition coefficient of phenylboronic acid and seven substituted derivatives. Four of of the compounds had a water/benzene partition coefficient between 1 and 6 and the other four had a coefficient > 50. The penetration of the compounds with the lower coefficients was seven-fold better than that of the other four. Marzulli *et al* (95), employing a series of organophosphorus compounds also found that the closer to unity the water/lipid solubility, the greater the rate of penetration. These results are in agreement with the opinion expressed by Hadgraft and Somers (1) that percutaneous absorption occurs optimally 'when the medicament combines lipid solubility with a moderate solubility in water'.

Chemical structure

In the three series of experiments mentioned, chemical structure did not appear to influence the rate of absorption but Scheuplein, Blank,

Brauner and McFarlane (119) showed that it has some relevance in the absorption of steroids.

The steroids were chosen to span as wide a range as possible in the presence of polar groups within a restricted range of molecular weights (oestrone mw 270.3 to hydrocortisone mw 360.4). Nevertheless, a thousand-fold difference between these two compounds was observed in permeability studies *in vitro* with human cadaver skin. Skin permeability of the other steroids in the series (progesterone, pregnenolone, hydroxypregnenolone, hydroxyprogesterone, cortexone, testosterone, cortexolone, corticosterone, cortisone, hydro-cortisone and aldosterone) were of an intermediate degree between that of oestrone and hydrocortisone. The rate of permeability in this series, as in the examples quoted in previous paragraphs, correlated with the lipid solubility of the compounds and was inversely related to the polarity. But the difference in lipid solubility between the extremes in this series of steroids was 50-fold which is in strong contrast to the 1 000-fold difference in permeability so that the authors could attribute only a small part of this difference to their lipid solubility. They postulated that the more polar molecules possessed a decreased mobility not only because they were less lipid soluble but also because of a stronger chemical binding with the stratum corneum. Thus, in the case of steroids, the chemical structure influenced to a significant extent the absorption through the stratum corneum. This possibility of a chemical binding with components of the stratum corneum may explain the formation of a corticosteroid 'reservoir' in this layer (120).

Chemical binding may also explain the failure of a series of synthetic anionic surfactants to penetrate the stratum corneum from low concentrations of aqueous solutions (121–123).

The slower rates of absorption of trivalent compared with hexavalent chromium at concentrations of 0.017–0.239 M (124) is also probably due to a difference in the ability of these two compounds to interact with the proteins of the stratum corneum. In the trivalent form, chromium binds with the proteins of the stratum corneum three times as much as its hexavalent form (125, 126). Hexavalent chromium is, however, reduced to the trivalent form in the skin (125) and it is thought likely that any Cr bound to the stratum corneum on the application of hexavalent compounds is largely in the trivalent form.

Molecular size

The percutaneous absorption of molecules much larger than those of

steroids was studied by Tregear (127), Kastin, Arimura and Schally (128) and by Iunin (129). According to these authors such macromolecules as colloidal sulphur, albumin, dextrans, polyvinyl pyrrolidone and polypeptides can penetrate the barrier readily if applied in solvents which possess a high lipid solubility, although they hardly penetrate at all if applied in an aqueous solvent.

The studies outlined above indicate that water and electrolytes exhibit the slowest rate of penetration through the stratum corneum. In the case of other compounds, the closer to unity the water/lipid partition coefficient the greater is their rate of percutaneous absorption. Chemical structure appears to be important because of its influence on the water lipid partition coefficient and on the interaction between test compound and stratum corneum. Molecular size does not appear to be relevant unless it is of macromolecular dimensions.

Solvents and vehicles

DMSO and other 'accelerants'

A variety of organic solvents are known to influence the percutaneous passage of chemical agents but few have been studied as intensively as dimethylsulphioxide (DMSO). It is a colourless liquid, and an excellent solvent for a variety of organic chemicals (130). Soon after the publication of its synthesis and of its physical properties it was realized that it had a great potential use in pharmacology because of the ease with which it traversed biological membranes (85, 131). Stoughton and Fritsch (130) investigated its effect on the percutaneous absorption *in vivo* of hexapyrronium bromide, naphazoline hydrochloride, flucinolone acetonide, and *in vitro* of ^{14}C hexopyrronium chloride and 4-^{14}C-hydrocortisone. Hexapyrronium bromide is a quaternary ammonium compound possessing anticholinergic properties and, dissolved in 95% alcohol, inhibits sweating at the site of application at concentrations of 0.2% and above. The addition of 20% DMSO to the solvent reduced the effective concentration to 0.008% thus increasing the potency by a factor of about 30-fold. A considerable increase in the pharmacological activity of naphazoline was observed by the addition of DMSO to the solvent. At a 0.04% concentration in 95% alcohol, no vasoconstriction and no pilo-erection was observed at the site of topical application in 16 volunteers. Presence of DMSO in concentration of 10%, 25% and 50% in the solvent produced a pharmacological effect in four out of 14, eight out of 24, 10 out of 14 treated subjects. To establish whether this

increase was due to an enhancement of percutaneous absorption these authors measured the passage of the ^{14}C-labelled compounds and found that the addition of DMSO increased absorption by a factor of 6.71 in the case of hydrocortisone and 27.4 in the case of hexopyrronium-methyl-^{14}C. Subsequent studies *in vitro* showed that DMSO not only enhances percutaneous absorption but promotes the formation of a steroid reservoir in human skin (132).

Comparative studies, *in vitro*, showed that DMSO is superior to other solvents both in enhancing penetration and in favouring dermal retention. This was clearly demonstrated in a study of the passage of ^{14}C-labelled griseofulvin, dissolved in neat DMSO, dimethylacetamide, dimethylformamide, ethanol or benzene, through human skin *in vitro*. Taking the rate of penetration of griseofulvin dissolved in benzene as unity, the ratios of penetration when the other compounds were used as solvents were 60, 40, 7 and 3 respectively. The superior property of DMSO to enhance percutaneous transit is seen even when its concentration is 50% in water. Thus, the ratios of penetration of ^{14}C hydrocortisone dissolved in 50% DMSO and in neat DMAC, DMFA and 95% alcohol was 20, 6, 4, 1. The retention of both griseofulvin and hydrocortisone in the excised skin was found to be roughly parallel to their rate of penetration and DMSO, neat or in 60% aqueous solution, was therefore superior to the other solvents in promoting skin penetration and retention. The retention ratios were approximately 25, 5, 5, 1 when DMSO, DMAC, DMFA or ethanol were used (133).

Similar results were obtained using other criteria for percutaneous absorption. After topical application of ^{14}C-labelled hydrocortisone or testosterone, 0.9% or 11.8% of the ^{14}C label appears in the urine in 5 days. Presence of 25% DMSO in the solvent increased the excretion rate of the label approximately four-fold. Dimethylformamide in the same concentration increased the penetration approximately two-fold while propylene glycol and mineral oil in 25% concentration slightly decreased penetration (134, 135).

DMSO was found effective in enhancing the percutaneous absorption of $HgCl_2$ *in vivo* in the guinea-pig. Using the 'disappearance measurements' technique, it was found that pretreating the exposure area with 1.0 ml of neat DMSO enhanced the absorption of $HgCl_2$ from a 0.239 M aqueous solution. No significant change in the rate of absorption was found at lower concentrations. In a similar experiment employing the same concentrations of mercury, the use of 1% soap or alkyl aryl sulphonate were as effective as DMSO (136).

The marked acceleration in the rate of percutaneous absorption produced by DMSO prompted a number of investigations into the local changes produced by this compound. This 'accelerant' effect is not thought to be due to increased skin circulation because this can be increased without increasing the penetration rate (115) or the skin 'clearance rate' (137). Furthermore, the accelerant effect can be observed *in vitro* with isolated non-perfused skin preparations (137). This *in vitro* effect was studied in detail by Sweeney, Downes and Matoltsy (138). Their results show that there was no significant change in the rate of passage of water through the skin when the epidermis was treated for 30 min with aqueous DMSO in concentrations up to 50%. At a concentration of 60% a two-fold increase was observed, at 80% and 90% the increase was 10-fold and 90-fold respectively. The changes in permeability in this study were irreversible and the authors concluded that permanent damage to the stratum corneum had resulted from this treatment. The fact that the concentration of DMSO was far more significant than the actual time of exposure in producing this effect was thought to be particularly relevant.

The *in vivo* work indicating a faster rate of absorption of corticosteroids dissolved in 25% DMSO appear to contradict the results of this *in vitro* experiment. It would seem possible, however, that the steroids and DMSO may have penetrated the stratum corneum because of a favourable water/lipid partition coefficient without producing damage.

Further *in vivo* studies failed to support the conclusion of Sweeney *et al* (138) that the damage produced by DMSO on the stratum corneum was irreversible. Exposure of the flexor aspect of mid-forearm to pure DMSO for 30 min in three volunteers increased the water loss 8-, 11- and 17-fold respectively but the effect was reversed within 6–8 h (139). This discrepancy may be a reflection of the ability of normal skin to repair the damaged stratum corneum.

Baker (139) attributed the effect of DMSO to its strongly hygroscopic properties so that its presence in the stratum corneum greatly increases the permeability properties by increasing the water content of this layer. However, the detailed studies of Allenby *et al* (137) indicate that profound chemical changes occur. DMSO extracted lipoproteins from the stratum corneum, an effect which is likely to disorganize its fibrillar structure. Electron-microscopic studies on the guinea-pig skin treated with neat DMSO show considerable structural damage (62).

Compared with the data available on DMSO, information on the mode of action of other 'accelerants' is sparse. Organic solvents, such as benzene,

ether and to a lesser extent, alcohol, have been shown to facilitate absorption of both water-soluble and lipid-soluble substances (107). According to Rothman (21) these solvents increase the permeability of the skin by 'attacking lipid building stones of the cell membrane'. Valette, Cavier and Savel (140) investigated another series of organic solvents with respect to their ability to enhance the percutaneous absorption of physiostigmine. The solvents belonged to eight classes of organic compounds: saturated aliphatic hydrocarbons, aromatic hydrocarbons, cyclic hydrocarbons, terpenes, primary saturated alcohols, ethylesters of saturated aliphatic acids and acetate esters of primary saturated alcohols. In this series, those solvents with the longer aliphatic chains were more efficient accelerants, while the presence of hydroxyl groups made the solvent less efficient. Baker (139) studied the effects of dimethylformamide and dimethylacetamide on the cutaneous barrier to water and thought that both these agents promoted percutaneous absorption by enhancing the state of hydration of the stratum corneum as a consequence of their hygroscopic properties. Allenby *et al* (137) compared the *in vitro* effect of a number of 'accelerants' on the penetration of ^{32}P-tri-*n*-propylphosphate (TPD), on the swelling of the skin and on its electrical conductivity. In this series, *iso*propanol, xylene, 0.9% NaCl in water, 8 M urea, methanol, chloroform, DMSO and phenol were studied. The most effective agents in promoting absorption of ^{32}P-TPD, in causing skin swelling and in reducing skin impedance were 8 M urea and DMSO, the least effective was *iso*propanol. All the substances which induced an accelerated absorption of ^{32}P-TPD extracted some organic component from the epidermis.

In previous studies, Elfbaum and Laden (141–143) using picrate ions showed that some accelerants cause swelling of the stratum corneum as well as increased penetration, while Vinson *et al* (60) and Montes *et al* (62) showed that accelerants may extract structural material from the stratum corneum. These results, according to Allenby *et al* (137) suggest that accelerants owe their effectiveness, at least in part, to their ability to lower the barrier properties of the stratum corneum by modifying its natural structure.

The ease with which lipids dissolve in the organic solvents mentioned might suggest that these increase the rate of percutaneous absorption by removing the lipids from the stratum corneum, and Szakall (144) showed that *in vivo*, defatting human skin by swabbing with ether for 3 min resulted in an increase in the amount of water absorbed lasting for 2 h. However, Winsor and Burch (145) have been unable to show an increase in the

permeability of excised human skin to water after the surface lipid film has been removed with lipid solvents. Blank and Gould (121, 122) failed to discover any increased permeability of the skin *in vitro* to sodium dodecyl sulphate after removal of lipids from the cutaneous surface by washing with acetone, ethyl alcohol or an ethyl alcohol–ethyl ether mixture for a for a brief period. If this period of washing was extended to 3–4 days permeability to sodium dodecyl sulphate and to sodium laurate was greatly increased. These results show that organic solvents remove lipids only after prolonged contact with the skin. In the experiments mentioned above, lasting at most 24 h, it would seem unlikely that the increased permeability observed is due to the removal of the skin lipid. The mechanism involved in the increase in permeability on removing the lipid in the stratum corneum by means of lipid solvents consists, according to Scheuplein and Ross (146), of 'hole formation and loss of water-binding capacity'. In the case of 'hydrogen-bonding solvents', for example DMSO and DMFA, the increased permeability is due to 'membrane expansion and uniform increase in media diffusivity' (146).

Surface active agents

Surface active agents differ considerably in their ability to penetrate the epidermal barrier, at least when low concentrations are employed.

The penetration of sodium laurate and sodium dodecyl sulphate was investigated by Blank and Gould (94) on excised human abdominal skin. They found that 20 h after application, sodium laurate had penetrated the epidermis and dermis from weak (0.005 M) unbuffered, mildly alkaline aqueous solutions. Sodium dodecyl sulphate (0.005 M) on the other hand had penetrated only in very small quantities below the barrier and for the most part was retained in the stratum corneum. This difference was attributed to a greater affinity of the skin proteins to sodium dodecyl sulphate and is in keeping with the observations (147–149) that alkyl sulphates and alkyl benzene sulphonates combine with proteins to an appreciable extent.

A difference between the *in vivo* rate of percutaneous absorption of *n*-dodecyl and *n*-hexadecyl sulphate in the rat was reported by Sprott (96). Using ^{35}S-labelled compounds in 0.65 mM concentration, the rate of excretion of ^{35}S in the urine after the application of *n*-hexadecyl ^{35}S-sulphate was twice that obtained following the application of *n*-dodecyl sulphate. Estimation of the amount of ^{35}S retained in the skin showed that the amount retained was inversely proportional to the amount absorbed.

According to Sprott (96) retention in the skin depends on the ability of the surfactant to interact with proteins; the greater this interaction the greater the amount retained locally and the less passes through.

At concentrations appreciably higher than those employed in the studies just mentioned, surfactants enhance percutaneous absorption. Studies by Sprott (96) indicated that surfactants promote the penetration of water and sodium iodide *in vivo*. Using tritiated water and ^{131}I sodium iodide he showed that pre-washing the shaved skin of rats with a bar of 'ordinary' soap increased the penetration rate three-fold. When the soap contained a high proportion of sulphonated fatty acids and fatty acid esters of isethionic acid, there was a four-fold increase in the rate of penetration. Similar increases in the rate of penetration of ^{131}iodide were observed after pretreatment of the skin with these two types of soap.

A similar enhancement of percutaneous absorption following application of surfactants was obtained in an earlier study (150, 151) on the influence of alkylaryl-sulphonates and soap on the percutaneous absorption of mercuric chloride and methyl mercury dicyandiamide. Both compounds were labelled with ^{203}Hg. The mercury compounds were dissolved in water, and in a 1% aqueous solution of soap and alkylaryl sulphonate, respectively. The presence of soap did not influence the absorption of the two mercury compounds. The presence of alkylaryl sulphonate increased the absorption of the organo-mercury compound and to a lesser extent that of mercuric chloride. These results confirmed other observations by these authors using the same mercury salts and surfactants (151). The addition of 0.2% sodium lauryl sulphate or 1.0% polysorbate 80 to solutions of chloramphenicol doubled the rate of penetration of this drug (98).

Even at lower concentrations, surfactants enhance percutaneous absorption. Using a 0.50 mM concentration of the sodium salts of C_8–C_{18} straight chain fatty acids, Sprott (96) found that the rate of penetration of tritiated water was increased with C_8–C_{14} fatty acid salts, the fatty acids with the shorter chain being the more effective. With a chain length of over C_{14} no effect on percutaneous absorption was observed. The sodium salts of alkyl aryl sulphonates at the same concentrations also increased percutaneous absorption. In this instance the longer the chain length the more effective was the compound. Bettley (45) correlated the surface activity of a series of ionic surfactants *in vitro* at 0.04 M concentration with their effect on permeability. He found that the potassium salts of caprylic acid and lauric acid, which depress surface tension to an almost equal degree, differed widely in their ability to enhance the permeability of the stratum

corneum to Na^+ and K^+ and also cetyltrimethyl ammonium bromide and sodium sulphosuccinate lauric mono-*iso*-propanolamide polyglycol, which show an equally moderate depression of surface tension, have very different effects on permeability.

Surfactants differ considerably in their ability to alter the permeability properties of the skin barrier. Unfortunately, the degree of lowering of the surface tension of water is not always given so that it is impossible to say to what extent this difference is related to the surface active property of the solution. The work of Bettley (45) would suggest that lowering the surface tension of water is not an important factor in enhancing skin permeability despite the possibility of removal of lipids when the surface tension of water is lowered. *In vitro* observations are obviously necessary before one can be certain on this point. The observations of Blank and Gould (94) and of Sprott (96) would indicate that the protein binding ability of the surfactant and the consequent alteration of the structure of the stratum corneum has to be taken into account as one of the factors that influence the alteration of the permeability of the skin. Recent studies (146, 152) tend to confirm the importance of protein denaturation in the increased permeability of skin induced by some surfactants.

Ointments, pastes and creams

Although the mechanisms by which individual organic solvents affect percutaneous absorption are amenable to investigation and data exist on many such solvents, the influence of formulations, containing a mixture of organic substances, on percutaneous absorption is less understood (153) and Williams (154) pointed out that there is no 'solid scientific evidence to guide the prescription of an active ingredient in . . . , an ointment, or paste or a cream'. It is equally difficult to predict with any degree of certainty the likely influence of formulations of this sort on percutaneous absorption of cosmetic chemicals. A limited amount of information is, however, available which could form the basis of some sort of a guideline.

The question of absorption of medicaments from ointment bases through the intact human skin was reviewed by Johnson and Lee (113). Many conflicting reports exist but in general most authorities considered that medicinals were absorbed more readily from animal and vegetable fats than from 'petroleum' bases. Experimentation in this field was hampered by the difficulty of accurately measuring the 'fatty materials and trace substances' in the tissues (113). The use of radio-isotopes considerably simplified these difficulties and Johnston and Lee (113) used NaCl, labelled

with a γ-emitting isotope of Na as a tracer in order to study its absorption from four types of ointment formulations applied to the forearm of human volunteers. The ointments were anhydrous lanolin, white petroleum jelly, lard, a 'hydrophile ointment base of the cholesterol type' and a 'washable' ointment consisting of cetyl alcohol, white wax, propylene glycol, sodium lauryl sulphate and 72% water. Observations over a 24 h period showed that absorption was best from anhydrous lanolin, next from lard, then from the hydrophile ointment and cetyl alcohol base and least of all from petroleum jelly. A better absorption of vitamin A in rats from an ointment containing lard than from one in which lard was substituted by petrolatum was reported by Sobel, Parnell, Burton, Sherman and Bradley (155). The more effective absorption of an electrolyte and an oil-soluble substance from lanolin and lard in these experiments, tended to confirm the reports of earlier workers.

The effects of ointment bases in the form of w/o and o/w emulsions on percutaneous absorption have been investigated. Shelmire (20, 156), using skin irritation as a criterion for the penetration through the stratum corneum, studied the effect of incorporating croton oil (5%), salicylic acid (2.5%), mercury bichloride (1.5%), resorcinol (5%) and ephedrine hydrochloride (2%) into petrolatum, a w/o emulsion, and o/w emulsion or *Carbowax 1500* (a water-soluble ointment made up of equal quantities of polyethylene glycol 300 and 2540) and left in contact with the skin for 3 h. The oil-soluble croton oil and ephedrine produced a maximum degree of irritation when incorporated in petrolatum. The intensity was less when the w/o emulsion was used as an ointment base and least of all when the o/w emulsion was used for this purpose. No irritation occurred when *Carbowax 1500* was used a sa base. On the other hand, the water-soluble substances salicylic acid, mercury bichloride or resorcinol were most effective when incorporated into the ointment base consisting of an o/w emulsion, other ointment bases gave a weak or no reaction. Similar results were obtained by Wahlberg (157) in his studies on the absorption of metallic test compounds from ointment bases.

Experiments with other compounds did not show such a clear distinction. Thus, Barrett, Hadgraft and Sarkany (158) showed that methyl nicotinate is absorbed equally well from an o/w or a w/o cream. Studies on the percutaneous absorption of betamethazone 17-valerate and of fluocinolone acetonide (153) showed that vasoconstriction (assessed from the area of pallor), produced by the preparation containing the valerate in *Carbowax 1500* was strikingly larger than that produced by

preparations in which the valerate was incorporated in aqueous cream BP, oily cream BP, and white soft paraffin BP. The reverse effect was found when fluocinolone acetonide was used in these vehicles instead of the valerate. The most marked vasoconstrictor effect was observed when fluocinolone acetonide was incorporated in aqueous cream BP and in the oily cream, it was less with white soft paraffin as an ointment base and markedly less with *Carbowax 1500*. Further work carried out by the authors (159) showed that the physical state of the steroid may have been responsible for this difference. Thus, using the microcrystalline rather than the coarse granular form improved skin absorption from white soft paraffin so that the degree of vasoconstriction produced was equal to that observed with the steroid incorporated in aqueous or oily cream BP. The improved absorption which accompanied this change in the physical state may be due to an improved contact with the skin surface since incorporating the steroid dissolved in 5% propylene glycol, thus improving still further skin contact, increased skin penetration from these two ointments and still further from white soft paraffin. Christie and Moore-Robinson (160) also found that fluclorolone acetonide produces a better vasoconstrictor effect when dissolved in propylene glycol and then incorporated into a 'petrolatum' ointment base. They found furthermore that the presence of 15% or 30% cetyl alcohol further increased the vasoconstrictor effect. According to the authors the addition of cetyl alcohol has the effect of increasing the concentration of the steroid in the propylene glycol and since it is this liquid phase that is likely to have the most intimate contact with the skin, enhanced penetration of the steroid is to be expected.

Experiments in which sodium salicylate or salicylic acid were applied to the intact rabbit skin in petrolatum USP XV or hydrated petrolatum USP XV provide further illustrations of the influence of the nature of the ointment on percutaneous absorption (161). 4–6 h after percutaneous application of petrolatum containing 6% salicylic acid, a concentration of 6 mg 100 ml^{-1} blood salicylic acid could be detected. When the hydrated ointment was used, the blood concentration reached a level of 9 mg 100 ml^{-1} blood. On substituting the sodium salt for the acid in these preparations, a peak concentration of only 1 mg 100 ml^{-1} could be detected in the blood. Addition of the surfactants, sorbitan monostearate, polyoxyethylene 20 or 40 sorbitan monostearate to petrolatum, enhanced the absorption of salicylic acid but did not affect that of sodium salicylate. Addition of these surfactants to the hydrophilic ointment reduced the absorption of salicylic acid but improved that of sodium salicylate. Salicylic acid is primarily an oil-soluble

compound while sodium salicylate is predominantly water soluble (161). The greater absorption of this acid from the hydrated ointment is probably a consequence of its greater affinity for the stratum corneum. The lower degree of absorption of the sodium salt is probably due to its much lower lipid solubility. The increased absorption of sodium salicylate from the hydrated ointment on the addition of surfactants is in conformity with their known ability to enhance percutaneous absorption when applied in an aqueous vehicle. The effects of surfactants on salicylic acid absorption is more difficult to explain. Salicylic acid has been shown to interact with substances containing polyoxyethylene groups (162) and this interaction may be expected to yield compounds with different solubilities, and consequently different rates of absorption.

So far only the physico-chemical interactions between the test substance and the constituents of the ointment have been considered in relation to their influence on percutaneous absorption of the active agents. Physiological factors are also involved. Baker (163) points out that the application of an ointment on the skin surface may lead to 'occlusion' of the skin surface so that the normal evaporation of water is prevented. This leads in turn to an increase in the water content of the stratum corneum and as a consequence to an increased permeability of this 'barrier' layer. In his experiments Baker (163) showed that the ointments differ considerably in their ability to achieve complete suppression of epidermal evaporation of water. Soft white paraffin with or without 5% propylene glycol was effective in this respect in the majority of patients tested. Ung. emulsificans BP and compound zinc paste achieved only partial suppression while anhydrous lanolin, and polyethylene glycol 1 500 failed completely in suppressing water evaporation.

The evidence reviewed indicates that the degree to which the test substance is soluble in the continuous phase is of primary importance since this determines the extent to which it comes into contact with the epidermis. Substances that are in solution in the dispersed phase do so only to a much more limited extent. Other factors of importance are the degree of partition between the continuous and dispersed phase and between these and the stratum corneum. The former eventually determines the available concentration at the skin/ointment interface while the latter influences to a considerable extent the passage of the test substances from the ointment into the skin. Ointments, pastes and creams may enhance percutaneous absorption by preventing evaporation of water from the stratum corneum thereby increasing its water content or its 'state of hydration'.

Sulphonamides do not appear to follow these general rules. Thus, the determination of the rate of penetration of sulphanilamide, sulphathiazole, sulphadiazine and sodium sulphacetamide applied to the shaved backs of guinea-pigs revealed that there was no difference in the rate of absorption when o/w or w/o emulsions were used (164, 165). Clearly, absorption of the compound from ointment bases is impossible to predict with any reasonable degree of accuracy. Only experimentation using the compound and the base intended for its incorporation can be of assistance in this respect.

'State of hydration' of stratum corneum

Observations on human beings have shown that conditions of high relative humidity enhance the damage produced to the skin by toxic chemicals. Cullumbine (18) and Kenshaw (166) observed that the vesicant properties of certain war gases was greater when the persons exposed were sweating freely. The influence of the water content of the stratum corneum on percutaneous absorption was studied in detail using both *in vitro* and *in vivo* techniques. Cronin and Stoughton (167) investigated the passage of ^{14}C-labelled ethylnicotinate through excised human skin supported over a chamber containing saline. They found that immersion of the skin in water of varying temperatures prior to mounting on the chamber resulted in a five-fold increase in the passage of ethyl nicotinate. *In vivo* experiments are in agreement with these observations. The concentration of topically applied ethylnicotinate necessary to produce a predetermined degree of erythema on the forearm of human volunteers was reduced by 5–10 times if the forearm was previously soaked in warm or cool water (168). Soaking of the forearm of human volunteers has also been shown to increase the percutaneous absorption of steroids. In a study (132, 169) ^{14}C-labelled trimethalone in 95% alcohol was applied to a small area of the forearm, allowed to remain for 30 min and then washed off with a standard washing procedure. Previous hydration of the arm resulted in a five-fold increase in the absorption of the glucocorticosteroid.

A more effective absorption of other steroids also takes place if they are applied under occlusive dressings. A suspicion that this might be so was expressed by clinicians who observed a better clinical response to topical glucocorticosteroids when the site of application was covered by a water-impermeable covering (170, 171). This suspicion was confirmed quantitatively (79, 80). Using graded concentrations of a glucocorticosteroid on

each arm, and covering one arm with an occlusive dressing while the other arm was protected by an elevated, perforated (non-occlusive) guard, it was found that the occluded arm showed vasoconstriction with glucocorticosteroid concentrations 100 times less than those required to give equivalent vasoconstrictor responses on the non-occluded arm.

A similar conclusion was reached by Scoggins and Kligman (172) in seven patients with dermatitis, using plasma cortisol levels as an index of the suppression of pituitary adrenal axis by the percutaneously absorbed synthetic steroids. The authors applied a weighed amount of a commercial cream containing a steroid mixture of known composition (prednisone, triamcinolone, fluocinolone acetonide, methylprednisone acetate and hydrocortisone) on areas of dermatitis and covered a number of these areas with an occlusive dressing, the rest were left uncovered. Under these conditions absorption of corticosteroids through diseased skin covered by an occlusive dressing is considerably higher than that from similarly affected but uncovered skin. The authors observed that: 'without an occlusive dressing, systemically significant amounts of these substances are absorbed only if the dose applied is very large'. Occlusion of the site of application in humans was found by Feldmann and Maibach (173) to increase 10-fold the amount of hydrocortisone absorbed.

In these experiments, occlusion of the site increased the percutaneous absorption irrespective of the type of vehicle employed.

No comments were made as to the possible mechanism responsible for the increased absorption from the occluded site. Covering the skin with an impermeable dressing prevents evaporation of sweat and of insensible perspiration resulting in an increased hydration of the stratum corneum. One would presume that this increase in the water content of the stratum corneum is responsible for the increased absorption in accordance with the results of the other authors already mentioned. The site at which water is retained in the stratum corneum is not clearly defined. Recent work (174) suggests that it is retained in the intercellular space in association with macromolecules possibly of a mucopolysaccharide nature.

Species variation

The rabbit, guinea-pig and rat are the most commonly used species for studying percutaneous absorption but the mouse and pig are used on occasions.

According to Draize (23) 'the rabbit is the most popular choice because it is generally more susceptible than man to the action of most substances'. This opinion was also expressed by Brown (34). He considered the rabbit more sensitive than other species and cited the work of Stevenson (175) with *Telodrin* in support. The percutaneous LD_{50} for a xylene solution of, *Telodrin* was 25 mg kg^{-1} in rats but only 5.8 mg kg^{-1} in rabbits. There are however, important exceptions to this general rule. For example, the percutaneous LD_{50} for parathion is lower for rats than it is for the rabbit (176).

Because of their low toxicity, it is doubtful whether the LD_{50} estimation is applicable to the cosmetic chemicals commonly in use. In any case, the wide difference in species sensitivity to toxic substances makes the LD_{50} an unreliable index of percutaneous absorption. The experiments conducted by McCreesh (48) in which he compared the sensitivity of a variety of species to a toxic dose of two organophosphorus compounds with their passage *in vitro* through the epidermis of the same species serves to illustrate this point. McCreesh (48) determined the percutaneous LD_{50} of two organophosphorus compounds (formula not given) in the rabbit, pig, dog, monkey, goat, cat, mouse and rat. He found that the rabbit and cat were the most sensitive followed in order by the dog, the goat, the monkey, the mouse, the pig and the rat. The dog, goat and monkey were about two to four times less sensitive than the rabbit and the cat, the mouse about six times, and the pig and rat about 10 and 20 times respectively. Measurement *in vitro* of the penetration of the labelled organophosphorus compound through the excised skin from dorsal thorax of these eight animal species revealed that there was no correlation between penetration and toxicity. The fastest rate of penetration was recorded in the case of rabbit and the rat, followed by that of the guinea-pig, cat and goat, monkey, dog and slowest of all, that of the pig. If one assumes the diffusion rate across the pig's skin to be unity, the rates of diffusion of the skin of the respective species are 9, 18, 18, 18, 27, 35, and 35 (rabbit and rat) times that of the pig.

For an objective assessment of the rate of absorption, *in vivo* tests are essential although valuable data may be obtained from *in vitro* work. A number of authors have conducted comparative tests of this sort. Employing the technique of measuring radio-activity in blood, urine and selected internal organs, Nörgaard (177) found that when 10 µl of an aqueous solution of ^{57}Ni was applied to the shaved skin of rabbits, and guinea-pigs, the degree of radio-activity after 24 h in the blood, kidney, and liver of the rabbit was approximately 5, 10 and 2 times those of the guinea-pig respectively. When ^{56}Co was applied in the same way it was found that the radio-

activity, measured 1–20 h after application, of the blood and urine from the rabbit were six to eight times greater than those of the guinea-pig. These experiments indicated that the rabbit skin was more permeable to Ni and Co ions than guinea-pig skin. This same author found that human skin was impermeable to cobalt and that both human and rabbit skin were impermeable to sodium.

The permeability of rabbit skin was also shown to be greater than that of guinea-pig in the work conducted by McDermot, Murray and Heggie (178). These authors applied a solution of a quaternary oxime (1-methyl-2-hydroximinomethylpyridinium methane sulphonate) in DMSO to the entire area of clipped skin (except head and legs) of rabbits and guinea-pigs. Hourly measurement of the plasma concentration of the oxime revealed a peak at 2 h in both species which was approximately 60 γ ml^{-1} in the case of rabbits and 40 γ ml^{-1} in the case of guinea-pigs. These differences are greater than they appear since the rabbit received approximately 0.5 g kg^{-1} of the oxime applied topically while the guinea-pigs received double this amount.

The relationship of the rate of absorption from human skin to that of rabbit or guinea-pig is not certain. According to Wahlberg (179) human skin is less permeable than that of the guinea-pig to sodium chromate, cobaltous chloride and mercuric chloride when tested *in vitro*. This author employed radio-active isotopes and calculated the mean absorption rates for labelled compounds. He found that the absorption of 0.034 M Na_2CO_3, 0.085 M $CoCl_2$ and 0.005, 0.239 M $HgCl_2$ was approximately three times greater through freshly excised guinea-pig skin compared to stored, human abdominal skin. The difference was less but still appreciable when freshly excised human skin was employed. Thus, the mean absorption rate for 0.08 M $HgCl_2$ was 1.8 times greater through freshly excised guinea-pig skin when compared with freshly excised human mammary skin.

If one excludes results from LD_{50} studies it would seem that percutaneous absorption takes place much more readily through skin of the rabbit than that of any of the other species studied. Guinea-pig skin appears to be less permeable than that of the rabbits and human skin is less permeable than either.

Active transport and metabolic transformation

According to Tregear (180) there is no evidence to support the presence of a system in the skin that actively transports water and electrolytes into

or out of mammalian organisms. The earlier claims by Folk and Peary (181) and Buettner (182) have been questioned by later workers (59, 180) on the grounds that insufficient attention was given to a number of physico-chemical factors which could account for the results observed by the earlier authors. No claims have been made that active transport affects the percutaneous absorption of other substances.

A striking example of metabolic transformation within the skin was reported by Fredriksson (54, 183) who showed that parathion (E605, diethyl 4-nitrophenylthionophosphate) is metabolized to paraoxon (E600 or diethyl 4-nitrophenylphosphate) which is then degraded to non-toxic metabolites within the skin of the cat. Another example of chemical transformation within the skin is the reduction of hexavalent chromium to the trivalent form (125). It is not known whether these examples represent enzymatic activity or are straight-forward chemical interactions.

Summary and conclusion

The principal cutaneous barrier to the absorption of substances from the external environment, in man and other mammals, is the stratum corneum. This barrier is broken by the ducts of sweat glands and hair follicles but percutaneous absorption via these appendages forms a small proportion of the total absorbed so that the major pathway for percutaneous absorption is across the stratum corneum. In fact, removal of the stratum corneum by the adhesive-tape stripping techniques results in a 10- to 12-fold increase in the absorption of any particular substance. Damaging the stratum corneum by abrasion or other means has a similar effect.

The stratum corneum allows some substances to pass through it more readily than others. The precise physico-chemical factors that determine this performance are imperfectly understood but the evidence accumulated from *in vivo* and *in vitro* work allows some broad generalizations to be formed. Lipids and lipid-soluble substances readily pass through the stratum corneum, organic compounds possessing hydrophilic groups do so less readily, while water and water-soluble substances, in particular if these are in an ionized form, traverse the stratum corneum with difficulty.

If the compound is applied topically in a vehicle, the rate of its absorption is considerably influenced by that of the vehicle, by the degree of its partition between the vehicle and the stratum corneum, and by its concentration in the vehicle. If the degree of partition is very small, the compound is absorbed along with the vehicle. If it is considerable, percu-

taneous absorption is probably independent of that of the vehicle. Concentration of the compounds in the vehicle appears to be important for aqueous solutions of electrolytes and less so for other types of compounds and vehicles. These features appear to be applicable to compounds in pure solvents or in creams and ointments.

The physiological state of the stratum corneum considerably influences percutaneous absorption. An increase in its temperature or in its normal water content considerably increases the rate at which substances, particularly water and water-soluble substances, pass across. Alteration of pH affects percutaneous absorption through an alteration of the state of ionization of the test substance or through damage induced by very high or low pH.

Measurement of percutaneous absorption has been made more sensitive and accurate by the employment of radio-isotope techniques. The older techniques employing chemical analysis and histochemical demonstration of topically applied substances are less widely used but may be of value in certain specific investigations. The method of 'disappearance measurement' of a compound, labelled with a suitable isotope, has found a wide application in experimental studies of percutaneous absorption *in vivo* but has only a limited application to human studies. This method needs to be combined with autoradiographic studies in order to allow for any error arising from stray (γ-emitters) or inaccessible (β-emitters) sources of radiation if the test compound is retained in the skin appendages. Excretion in urine, faeces and expired air of the topically applied compound or its metabolite provide a good qualitative evidence of percutaneous absorption but with few exceptions are a poor guide for quantitative studies. The same comment may be applied to biochemical estimations (such as enzyme levels), measurement of storage in internal organs and assessment of the percutaneous LD_{50}. Despite this reservation such tests may provide invaluable information in some fields of toxicity studies, e.g. pesticides.

The pharmacological effects chiefly employed in human percutaneous studies are vasomotor effects and inhibition or stimulation of sweat production under physiological conditions. *In vitro* studies employ both chemical and isotopic techniques for measuring the transepidermal passage of a particular test compound on skin samples removed from animals or man and mounted on a suitable chamber. These techniques give useful comparative information on the percutaneous absorption of different chemicals or the same chemical in different solvents. They also provide basic information on physiological and pathological skin conditions which affect trans-

epidermal absorption. However, the results from *in vitro* studies cannot be extrapolated to the *in vivo* situation, but they do provide some guide to the design of *in vivo* experiments.

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