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# A BIOLOGICAL BRIEF ON PERCUTANEOUS ABSORPTION

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## Historical

From the earliest times physicians have sought to treat man's melange of health-destroying maladies by applying medicaments to the skin. A wonderful variety of ointments, pastes, plasters and complex inunctions were created for every kind of disease.

With the advent of scientific medicine in the last half of the 19th century, this route of administration fell out of favor. It became obvious that no matter how hard one rubbed galenical mixtures over the entire body, there was little or no pharmacologic response compared to oral drugs which at least could make people sick if not well!

In 1877, Fleischer declared that the skin was totally impermeable, a complete shield against the external world (1). This extreme view could not hold for long; after all dermatologists were more or less successfully treating syphilis by rubbing salts of mercury over the skin. By the turn of the century Schwenkenbecker (2) perceived that the skin would admit some substances much better than others. It was then seen to be semi-permeable. Simple experiments using clear cut endpoints such as death of the animal led to useful generalizations. It could be shown that lipid soluble agents were more likely to penetrate than water soluble ones, a





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rule which still holds. Moreover, the base form of alkaloids could kill animals by topical application but the salts were innocuous. For example, strychnine was lethal but strychnine chloride was innocuous. It is still a useful generalization that polar electrolytes and ionized salts penetrate very poorly. Nonetheless, permeability is too complex to admit of easy generalizations, a habit which some current authorities find difficult to control. Thus, the statement that penetration rates of diverse chemicals are correlatable with their degree of lipid solubility is simplistic. Too many agents do not show a nice correlation. No single physical datum predicts penetration behavior. Even crude approximations are hazardous. For a long time, it was believed that flux could be predicted on the basis of ether:water solubility partition coefficients, a ratio of one being considered most favorable (3,4). For limited classes of molecules this rule sometimes holds. For many others, it is inapplicable. Still, one can always find a germ of wheat among the chaff. As a rule, substances which are soluble both in water and in lipid solvents are more likely to enter skin. This is not to say that it will be impossible to predict flux from analysis of physico-chemical constants, such as the vehicle: skin partition coefficient, diffusion constants, etc. Indeed, within certain classes of molecules, steroids for example, that bright day has already dawned (5). For a cheerful view of the future based on tight mathematical analyses, the scholarly review of Dugard is highly recommended (6).

The modern foundation for understanding the principles of percutaneous absorption has been built on two pillars: (1) measurement of flux in vitro using samples of skin mounted on diffusion chambers; (2) the availability of tritium and carbon radio-labelled materials, improving the exactitude of measurements in vitro and in vivo.

Some lecturers decry the paucity of hard data on percutaneous absorption. I, on the contrary, am impressed with its plentifulness. At this very time, theory is replacing empiricism and drugs are being synthesized which will possess specific characteristics favoring their penetration into skin.

Knowledge of percutaneous absorption has fostered the brilliant development of transdermal therapeutic systems, a realty today. This achievement results from the integrated efforts of engineers, physical chemists, biochemists and pharmacologists. The prospects before us are thrilling.

Still, there are plenty of difficulties to tussle with. The early estimates were basically correct--the skin is not very permeable. It is an impressive seal. We do not dry out nor do vital substances leak out. Most exogenous materials are effectively kept out. What is the secret of the skin's imperviousness? Clinicians have long suspected that the obstacle to penetration must be located superficially. How, for example, could a blister form if the epidermis were permeable to water? For elegant simplicity the illuminating experiments of Monash are hard to beat (7). He found it took hours to secure loss of sensation by topical application of anesthetic agents. However, after stripping repeatedly with Scotch tape, the site became numb in a few minutes. This was indisputable proof of a superficially located barrier since Scotch tape removes only the dead horny cells of the stratum corneum. But, note the date for this revelation, 1957!

No one has been more ingenious than Stoughton in using simple methods to explore the permeability behavior of human skin (8,9). He has brought to light many important facets by applying substances which cause some rapid physiologic display, such as blanching (vasoconstriction), reddening (vasodilation) or sweating. By intradermal injection, these responses could be elicited with concentrations of $10^{-6}$ to $10^{-7}$. By contrast, concentrations orders of magnitude greater ($10^{-2}$) were required topically. Clearly, the epidermis is a substantial structural obstacle for all these reactions are mediated by blood vessels situated in the dermis.

Many have labored in this field for over a century; yet substantive knowledge is astonishingly recent. The most important efforts have focussed on establishing a theoretical foundation. The most notable works are by Tregear (10), Blank (11) and the modern master, Scheuplein (12). These last

two, who collaborated for a decade at Harvard have brilliantly illuminated the subject (13). None are dermatologists. It is a bit awkward to have to say that certain skin doctors who have set sail on these settled waters seem to have been blown off course (see below).

It is edifying to review the barriers that had to be vaulted before some understanding of the "barrier" could be reached. Arguments had raged for more than half a century. It is ironical that morphology, the bed rock of skin science, was a treacherous swamp. Appearances turned out to be deceiving.

The dispute centered around localization of the barrier. It was close to the surface but exactly where and what was it? In 1951 Berenson and Burch compared transepidermal water loss through isolated sheets of whole epidermis and its stratum corneum (14). The results were unequivocal; only the horny layer was important. But there was no consensus regarding its anatomic construction.

One can quote the highest authorities to illustrate how profoundly its structure was misperceived.

The maestro of cutaneous physiology, Rothman, could state as recently as 1954: "In mammals, the horny layer is shed in visible small particles, involving the disintegration of keratin fibrils into minute microcrystals on the surface; the shedding cannot be seen microscopically because of the submicroscopic size of the desquamating particles" (15).

In other words, the horny layer was not comprised of cells but of fibrils; it was amorphous, a proteinaceous matrix.

This theme was repeated again in 1964 by Malkinson: "The upper layers of the stratum corneum cannot be considered a barrier since they are composed of a coarse and porous network of keratin fibers which are readily penetrated by anions and cations and even by molecular aggregates" (16). So, one had to look more deeply and unfortunately backwards. In 1924, Rein had postulated

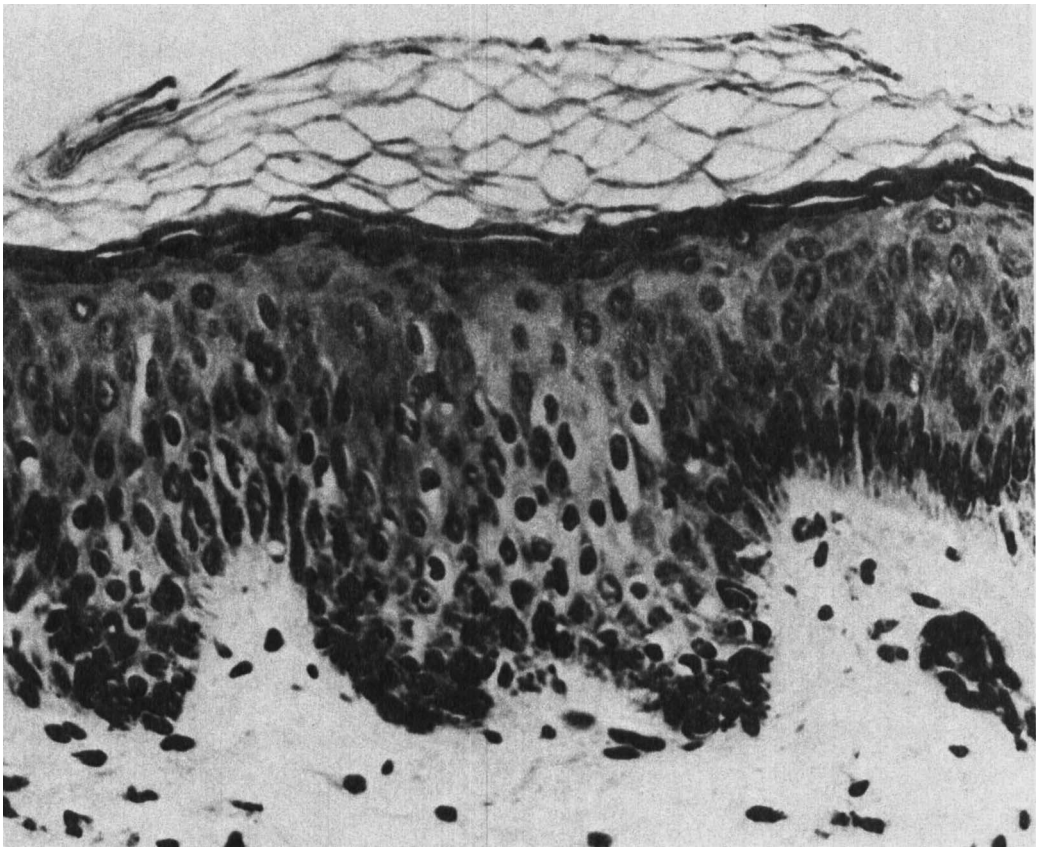
an electrically charged membrane at the base of the horny layer (17). This membrane was a good fit, at least geometrically. It was hailed as "the" barrier. Most studies were consistent with this idea. In 1945, Mackee, Sulzberger and co-workers observed histologically that topically applied dyes penetrated the outer layer easily, suddenly stopping at the base of the horny layer (18). Later, the electronmicroscope could be marshalled in favor of locating the "barrier" at the base of the horny layer where a distinct thin membrane could be observed (19).

Final "proof" came with I. Blank's famous in vitro studies with abdominal skin (20). He removed layer after layer of horny cells with tape, measuring transepidermal water loss after each stripping. Little happened until he reached the bottom of the horny layer. The curve suddenly shot upwards as the last few layers were removed. The "barrier" really was at the base of the horny layer. Investigators set about to isolate it anatomically. Szakall thought he had succeeded by hydrating the skin before stripping. He could sometimes obtain a thin sheet (21). Szakall even went so far as to provide information on its chemical composition declaring that it was also responsible for the water holding attributes of skin. All these ideas were wrong, as persuasive as they seemed. Szakall's membrane is the stratum lucidum, the "glistening" layer one reaches by stripping. It is usually left behind and has no barrier properties whatever. Blank's observations have never been confirmed.

By looking at a transverse section of skin we can actually "see" why these notions were so appealing. The horny layer seems acellular and spongy (fig. 1). Histologists to this day describe it as a basket weave of thin lamellae separated by empty spaces. Its porosity is "obvious".

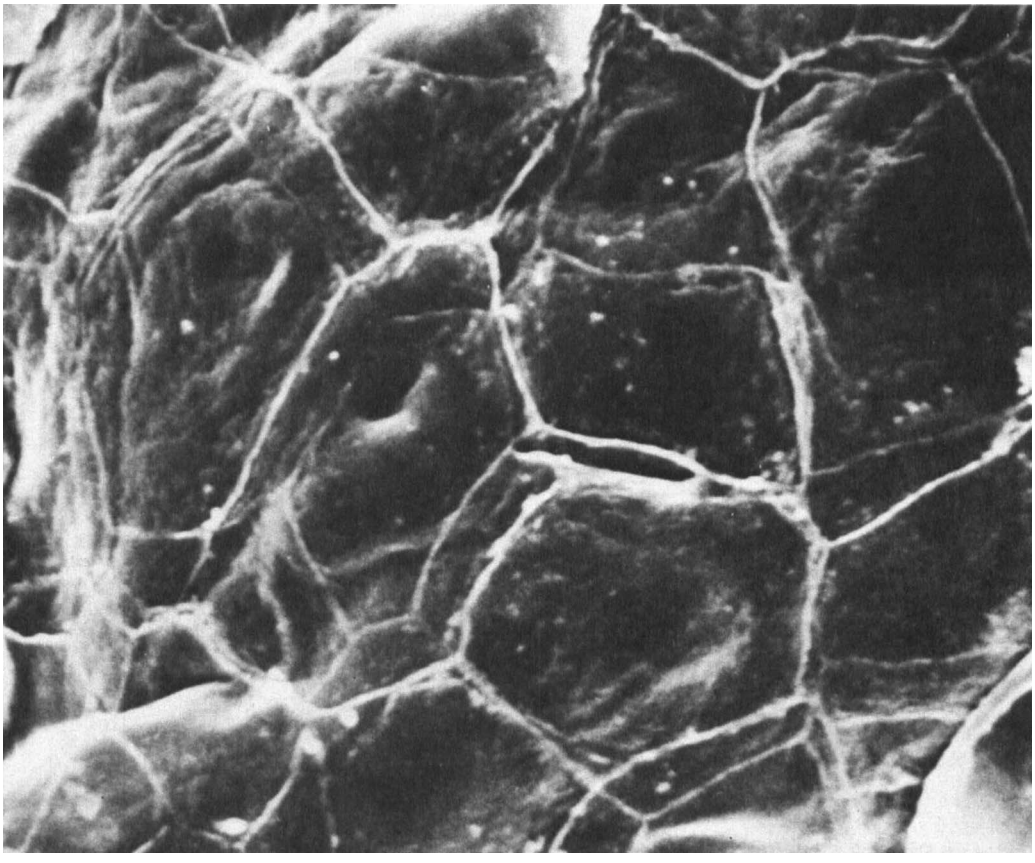
This image is artefact, an enormous distortion of reality resulting from the injuries of histologic processing (22). The "spaces" are created by fixation and dehydration, corrupting the tissue beyond recognition.

With a modicum of care one can isolate the horny layer intact. Immersing whole skin at 60°C for one minute enables one to peel off the epidermis. The



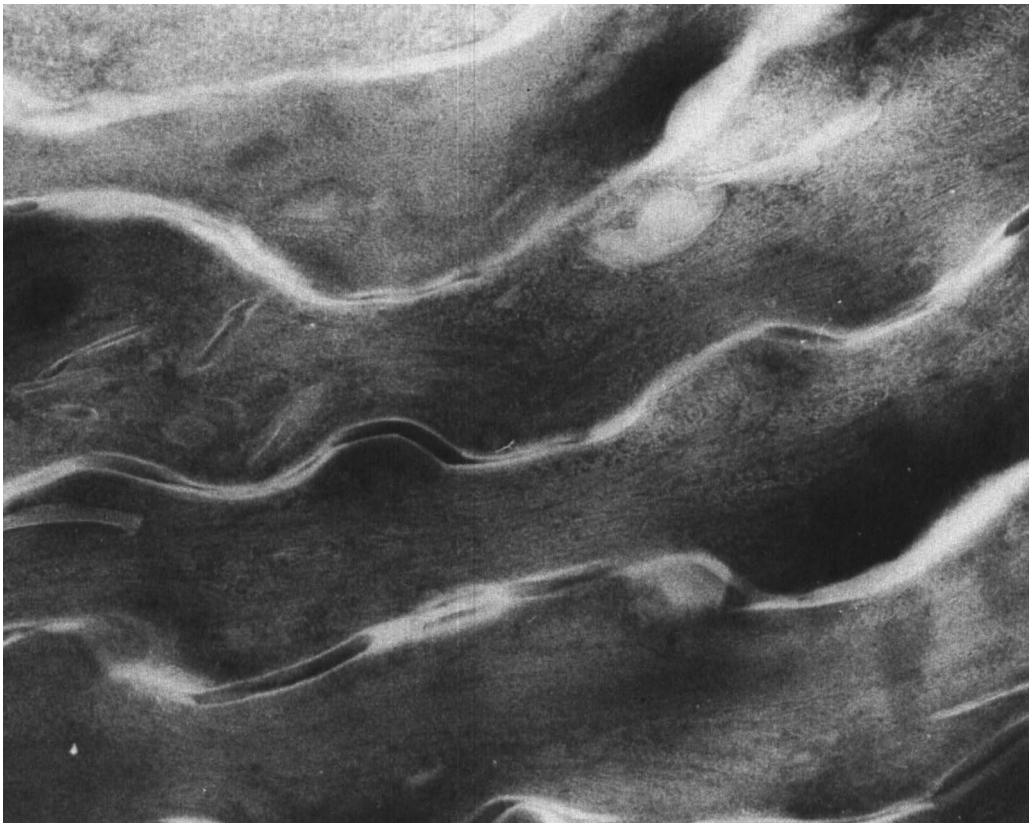
1. Human epidermis by light microscopy. The outermost layer, the stratum corneum, seems to be made up of thin lamellae in a basket-weave pattern. The large spaces suggest porosity. This appearance is all artifact. The lamellae are really flattened cells which in real life are packed together to form a coherent membrane (H & E, X 50).

underlying epidermal cells can then be scraped away releasing the stratum corneum in its pristine glory (23). Its true form is that of a transparent, tough, coherent membrane. By viewing it perpendicularly to the surface one can observe that it is made up of polygonal cells that are closely joined (fig. 2). The cell walls are so sturdily constructed that strong alkalis lyse the cell contents without disrupting them. The stratum corneum is



2. Scanning electronmicroscopic view of the surface. Flattened polygonal cells form a continuous sheet without spaces. The cells tightly overlap each other to form the "barrier".

markedly hygroscopic and viscoelastic. When immersed in water, it swells to about three times its original width, absorbing 4 to 5 times its own weight. When not wet it is about 10 microns thick, into which about 15 layers of flattened, horny cells are compressed. The cells are densely packed with fibrous protein (keratin), in the form of filaments embedded in an amorphous matrix (fig. 3). This design results in a strongly impervious structure that is nonetheless flexible enough to accommodate to every movement without splitting or cracking. It is well to keep in mind that it is not homogeneous like a sheet of plastic. It is distressing that many writers still refer to the horny layer as "keratin" as if it were a homogeneous acellular polymer.



3. Cross section of horny layer by electronmicroscopy. The cells are densely packed with keratin fibrils and are separated from each other by narrow spaces. The dark intercellular discs are the remnants of desmosomes which in the living epidermis are sites of attachment. Despite appearances, the intercellular spaces are not empty.

It is unimpeachable doctrine that it is the horny layer which restricts water loss and is the chief impediment to the inward diffusion of chemicals. It is the rate limiting membrane. For all practical purposes, the structures beneath, the viable epidermis and dermis can be disregarded. Their diffusivity is orders of magnitude less than the stratum corneum. The horny layer virtually excludes electrolytes such as sodium chloride and simple salts. When it is removed, even these molecules easily enter the epidermis and dermis.

The latter offer trivial resistance to the passage of varied chemicals, provided these do not bind to the substrate. This has been shown time and time again (13).

Further, it is established beyond doubt that transport across the stratum corneum does not depend on metabolic processes. Diffusion is a purely passive process. Accordingly, the stratum corneum obeys the physico-chemical laws of diffusion across the membranes. The mathematical embodiment of transport of substances into skin is Ficks law, which holds strictly. Its expanded version covers all instances as described in detail by Dugard (6).

Some dermatologists find this emphasis on the stratum corneum discomforting and raise up various objections. For example, Winklemann, an eminent student of skin, keenly dislikes the idea (24). In his view the "approach which considers the epidermal barrier passive and inanimate discounts the many such variations of epithelial and mesenchymal structure which undoubtedly have some role in the passage of material through the skin". He reminds us that the epidermis is alive, continuously renewing itself even capable of phagocytosing (ingesting) India ink, cholesterol, and other large aggregates which could perhaps help move material away from the surface and "thus be an important feature of percutaneous absorption". These views are, to say the least, quixotic. They are also immaterial and irrelevant. Winklemann (and he is not alone) are vitalists to the end, refusing to surrender to the bloodless physical chemists.

Maibach and co-workers have published extensively on the penetration of various drugs and chemicals through human skin in vivo (25). In their comprehensive review titled "ten steps to percutaneous absorption" one again discerns unwillingness to accept the decisive role of the horny layer (26). In an earlier study of the penetration of hydrocortisone through stripped living skin, Maibach was surprised to find only about a four-fold increase (27). Under an impermeable dressing on normal skin, absorption was increased ten-fold, suggesting that the horny layer may have been over-rated as a barrier. I see no reason for doubting the accepted doctrine since careful

in vitro measurements always end in emphasizing the decisive role of the horny layer. Of course, the underlying structures take up space and have to be traversed. The key point, however, is that their resistance is hundreds to thousands of times less than that of the horny layer, negligible in any practical sense.

In my opinion, experimental error was responsible for the finding that stripping was less than half as effective as hydration and did not make the skin freely permeable of hydrocortisone. Stripping is a damaging procedure allowing leakage of serum which, unless covered, results in a dry crust. This alters the surface drastically. Depositing a small amount (4 $\mu g/cm^2$) of corticoid from an ethanolic solution could result in sequestration of crystals. Indeed, penetration increased twenty times when the stripped site was occluded. I regret to labor the issue but there can be no compromising on principles which are based on solid observations. Indeed, Malkinson monitored the disappearance of radiolabelled hydrocortisone applied to stripped skin. As expected, penetration increased several hundred-fold (28). The observations of Shaw and co-workers are even more telling because the methodology was so rigorous (29). With scopolamine free base they found steady-state flux to be about 6.0 $mg/cm^2/hr$ through either separated epidermis or whole skin whereas through dermis alone the value was 1342, more than 200 times greater. Theory is satisfied. This thesis has been proved to the point of boredom (30).

It is a valid generalization to state that the stratum corneum allows no substance to penetrate easily and allows all substances to enter slightly. With the sensitive methods now available, trace amounts can be detected, forcing us to add qualifications to the degree that electrolytes cannot penetrate. Thus, Wahlberg could show some penetration of radiolabelled sodium chloride through animal skin (31). Clinicians can easily accept the notion that the barrier to salt electrolytes cannot be absolute. After all nickel and chromium are quite common contact sensitizers, and must have the capacity to get through normal skin from their various salt forms. Patch testing of the normal skin of sensitized persons proves this. Likewise,

substances of extremely high molecular weight such as proteins are virtually excluded yet, topically applied tuberculin can elicit a positive reaction in sensitive subjects. Certain food antigens can elicit wheals. The important point is that there may be as much as 10,000-fold difference in the flux of different substances (32). Of course, chemicals which grossly damage the horny layer destroy this selectivity. In that case differences in rates of diffusion may nearly disappear. The action of certain "accelerants" such as DMSO is partly explicable by structural injury to the barrier. The attitudes of dermatologists may have been unduly biased by their understandable pre-occupation with abnormal, lesional skin. For example, in inflammatory dermatoses such as psoriasis the diseased skin is far more permeable to water and to chemicals (16).

Is the horny layer homogeneous from top to bottom? Does every cell layer contribute equally to diffusional resistance? These are legitimate questions. Previously, the imaginary barrier at the base of the horny layer was called the stratum conjunctum while all the loose stuff above was the stratum dysjunctum. The terms are archaic now but may be worth rehabilitating if we modify their original meaning. Except for the outermost portion where horny cells are being shed in tiny flakes (the desquamating zone), every cell layer in the underlying compact zone is equal to every other one in diffusional resistance. The sloughing, loose, surface layer is only 2 to 3 cell layers thick and is easily penetrated, even by particles as large as bacteria or bentonite. This newly defined stratum dysjunctum is a negligible barrier. Everything below this is the stratum compactum where the cells are tightly bonded, forming a coherent membrane. Substances diffusing through the compactum show the concentration gradients predicted by theory. For example, Zesch and Schaeffer applied radiolabelled chemicals to the surface and after about 1.5 hours analyzed the amount of tracer removed by successive strippings (33). The largest amount came off in the first 3 strips (the stratum dysjunctum); thereafter the amount of label fell linearly with increasing depth. This simply has to be the case. It is worth noting that fewer and

fewer cells come off with serial stripping perhaps indicating tighter intercellular connections among the younger cells below. Zesch and Schaeffer wisely took this into account and related the amount of radio-labelled tracer to the weight of horny material removed per strip.

Curiously, Klaschka, a dermatologist, put the stratum compactum on the outside and the dysjunctum below (34). This is an extraordinary position, incompatible with all other observations.

## The Modern Scene

Why should we worry ourselves about the intricacies of the barrier? The advanced state of technology compells this. In simplier times, we were comfortably encased in our impermeable skin, safe from external noxae. The wizardly of the synthetic chemists has changed all this. The horny layer, to be sure, is admirably constructed and its reputation for imperviousness holds. But thousands and thousands of new molecules are being synthesized annually, many so potent that absorption of tiny amounts may produce systemic toxic effects, even death. For example, it takes only a few micrograms of certain cholinesterase - inhibitors to kill a man; lethal amounts can cross the barrier after brief contact with a limited area of skin. It is no wonder that the military have supported basic work on percutaneous absorption!

Toxicologists can cite numerous instances of adverse systemic effects from topically absorbed materials. These poisonings are generally accidental, mostly related to occupational and industrial usages. Very well known in this regard are the toxic effects from spraying organo-phosphorus insecticides, such as parathion (35). Other compounds which can poison man through his skin include chlorinated hydrocarbons, aromatic amines, dinithrophenol, etc. (36).

These happenings have ended our security. Instead of viewing the skin as essentially impervious, we must now view it as a potential portal of entry.

Often, it has taken disaster to drive this lesson home. The hexachlorophene tragedy bears witness to this; that saga is a valuable object lesson (37). Many thousands of persons had applied hexachlorophene in multitudes of products without a whisper of toxicity for two decades. Then suddenly, French infants who had been dusted with hexachlorophene-containing talc died. The lethal combination was: (1) the infants were premature; there is evidence that the barrier is incomplete until shortly before birth. (2) the hexachlorophene was applied to the diaper area which is always wet and warm, circumstances which greatly promote penetration. Hexachlorophene was known to be substantive to skin and rather toxic intraperitoneally but up to then there was confidence in the barrier. Complacency if that kind will never return. Salicylic acid and phenol are time honored ingredients of diverse medical topicals. We need to be reminded that these can cause serious toxicity, death too (38). Some ichthyotics, with their imperfect barriers, are being doused daily with solutions of salicylic acid to remove scales. The salicylism which results usually goes unrecognized.

Nowadays, toxicologists are likely to spot toxic substances before widespread exposure occurs. Disaster was averted when certain cyclosiloxanes, intended for incorporation in cosmetic creams, were found to be topically gonadotoxic to animals (3). Subacute dermal toxicity is a required step in evaluating new chemicals. The animals are monitored clinically as well as by histologic examination of important organs such as the brain, liver, gonads, kidney, etc. A striking recent example of the protection provided consumers by professional toxicologists is the experience with acetyl ethyl tetramethyl tetralin (AETT). During routine sub-chronic dermal toxicity studies with a new fragrance it was observed that the skin of the rats had turned blue. The brain was similarly discolored. The individual components were then separately evaluated leading to the identification of AETT as the culprit. It finally turned out that AETT was a rather potent neurotoxin that could severely damage the brain and peripheral nerves. A 1% ethanolic solution was neurotoxic to two-thirds of the

of the animals when applied repeatedly. Of course, AETT was voluntarily banned. One has to be constantly on guard against complacency. For example, clindamycin is a topical acne treatment. Blood levels are not detectable from 2% solutions applied to the face. Nonetheless, diarrhoea and colitis have been reported, rare as these may be. Application to hamster skin is lethal. Colitis results from the selective overgrowth in the gut of a toxicogenic stain of Clostridium (41). In toxicology, almost anything can happen no matter how unlikely.

Toxicity is only one side of the permeability coin. The other has a brighter imprint, namely modeling molecules to enhance their therapeutic efficacy. The creation of increasingly potent topical corticosteriods is a brilliant example of designing drugs to achieve predetermined goals. Penetration can be enhanced by modifying the surface microenvironment. The best known example is the simple device of covering the treated site with an impermeable plastic film, usually Saran Wrap. Sulzberger and Witten discovered that the therapeutic activity of hydrocortisone was enhanced under an occlusive dressing (42). This was not well understood at the time but occlusion became an important tactic in treating refractory skin disease.

The dormant event in occlusion is hydration. This swells the horny layer making it more permeable to lipid soluble molecules which already possess a capacity for penetration. Occlusion does not facilitate absorption of polar electrolytes. Water also becomes part of the membrane and increases its diffusivity, a reversible process after drying out. McKenzie and Stoughton found that the penetration of corticosteroids could be increased a hundred-fold just by covering the skin by an impermeable dressing (43). By applying steroids in a volatile solvent like acetone and simply covering the donor receptable to increase humidity, Scheuplein and Ross showed that flux was sharply increased (44). A secondary factor in occlusion is raising the surface temperature from about 32°C to 37°C, adding thermodynamic drive. As expected, diffusion is temperature dependent and accordingly can be increased or decreased.

Therapeutic enthusiasm must always be tempered since enhanced potency, however achieved, will almost always be accompanied by enhanced toxicity. Indeed, a single 15 gram application of clobetasol propionate, the winner of the steroid olympics, will quickly cause complete inhibition of cortisol secretion by the adrenal gland. The systemic side effects from injudicious use of topical steroids are numerous and serious, including adrenal failure, Cushing's syndrome, etc. (45). The advance to be looked for next is not the creation of more potent steroids. The new ideal will be an anti-inflammatory agent whose efforts are limited to the skin by virtue of metabolic inactivation during the process of absorption. No dream this. The era of pharmacokinetic tailoring is already upon us.

Topical therapy has obvious advantages, putting the drug directly where it is required without perfusing vital organs. Innovative delivery systems are providing new opportunities. For example, the oral administration of psoralens along with ultraviolet light is an effective treatment for intractable psoriasis. Blood levels comparable to oral administration can be achieved by putting psoriatics in a bath of dilute psoralen for a short time. This is expected of a rapidly penetrating lipid-soluble drug. The amount absorbed is, of course, proportional to surface area which is maximal in the bathtub, about 2 square meters. Also, by restricting the time of exposure, the drug preferentially diffuses into lesional skin whose barrier is, of course, defective! This results in a relative sparing of the normal, uninvolved skin from the phototoxic effects of psoralen, a known photocarcinogen. Finally, water enhances penetration through its hydrating effect.

It is not only dermatologic patients who are benefiting from increased understanding of cutaneous permeability. Using the skin as a portal of entry, opens up exciting possibilities to the internists, especially for drugs which exert their effects at low blood levels. To appreciate this fully one has to contrast the kinetics of oral and cutaneous absorption.

Drugs absorbed in the gut give a rapidly rising blood level usually followed by a sharp decline. These fluctations are far from optimal since

the tissue concentration will sometimes be greater than required and at other times smaller.

By contrast topical administration tends to dampen "pulse" effects. Instead, a lag period is followed by a slower rise to a peak blood level followed by a slow decay. By approximately scheduling the applications, steady state levels can be approximated. Because of its relative impermeability, the skin functions as a depot which slowly releases the drug into the circulation. The kinetics have to be worked out specifically for each drug. Not all drugs can form skin depots, especially these which are rapidly absorbed. Still, the synthetic chemists can modify drugs practically at will. For example, we know that the undissociated form of a molecule is far more likely to be absorbed than in the ionized state. Wadderburn found that at pH 2.0, almost 100% of benzoic acid was absorbed whole at pH 6, less than 2% was absorbed because of dissociation (46). A similar explanation applies to the greatly enhanced penetration of scopolamine at pH 9.6 in comparison pH 4.0 (29). At the higher pH the ionized salt is converted to the base, which is also more lipophilic.

Transdermal delivery systems represent the reduction of these ideas to therapeutic practice. Nitroglycerine occupies the spot light now but there are many other drug candidates in the wings. Nitroglycerine ointment for angina was a useful product, a creative development for its time. This was the precursor of transdermal systems and had a sound theoretical foundation, though less elegant and not so controllable. Also, delivering drugs via the skin avoids the first pass inactivation by the liver from the portal circulation.

In designing transdermal devices, it seems to be undecided whether the horny layer should be the rate limiting membrane or whether the device itself should be controlling. In the scopolamine transdermal system, the device is set to deliver the drug at a rate below maximum capacity of the horny layer. In making decisions of this sort, one must take into account the sometimes great individual differences in permeability, a reflection of variability of the horny layer barrier. For example, Marzulli found that absorption from the

most permeable region (post-auricular) in some persons was similar to the least permeable (plantar) in others (47). A clinical correlate of this is the substantial individual differences in susceptibility to irritating chemicals. Frosch and Kligman found that the time to raise a blister to ammonium hydroxide varied from 5 to 30 minutes. Moreover, then established that the blistering time was directly proportional to the number of horny cell layers (48). Other studies in our laboratory support the generalization that the horny layer mainly accounts for differences in susceptibility to diverse irritants. These findings are consistent with Ficks law which states that flux varies inversely with thickness of the membrane. It has been shown by direct measurements that the thicker the horny layer, the less the permeability (46,49). However, thickness is not an absolute; the quality of the horny layer must also be considered. This applies to normal skin of different regions.

Those who write reviews often misunderstand this. Grasso and Lansdown, for instance, point out that the palms and soles are far less permeable than other body regions (50). They attribute this to the many-fold greater thickness of the horny layer. But then go on to say that this doesn't seem to hold in vivo because Maibach et al had observed that penetration of organo-phosphorus insecticides occurred just as readily through the palms as the forearms (51). The fact is that for equal thicknesses the only valid comparison, the palms and soles are far more permeable than forearm skin. Though 40 times as thick, transepidermal water loss through the palms in vivo (in the absence of sweating) is considerably greater than elsewhere (13). Qualitatively, the palmar "barrier" is quite inferior because of the way it is constructed. Its horny cells are spheroidal rather than flat plates; they are also less densely packed with fibrils. Its purpose is mechanical protection, not chemical imperviousness. It is all to easy to be fooled. For example, topical drugs which induce erythema or blanching elsewhere do not evoke these responses on the palms even with high concentrations. This is often used to illustrate the high impermeability of this region. Nonetheless, this interpretation is false. First of all the lag time is an exponential function and would be inordinately long. More importantly, the difference in concentration on both sides of the membrane is thickness

dependent. The further molecules have to go, the less the concentration in viable tissue. Thus, although absorption may be greater on the palms the level of drug in the vicinity of blood vessels is too low to cause vasodilation or vasoconstriction. These paradoxes and confusions would be lessened if one used permeability constants to describe penetration behavior, these automatically takes thickness into account. This further emphasizes the importance of in vitro measurements (see below).

## The Horny Layer Reservoir

Mid-twentieth century seems to have been a time for seminal observations. In 1954 Guillot found that urinary excretion of salicylic acid lasted for days after a single topical application (52). After intradermal injection excretion was complete within 24 hours. This was the first demonstration of a depot or reservoir in the superficial skin. However, it was a British dermatologist, C.F. Vickers who fully comprehended the reservoir concept and worked out its operational details. His review of the subject is an engaging classic (53).

To appreciate the concept, one must recall that the horny layer is dense, multilayered and extremely compact. It contains most of the mass of the epidermis. Substances sorbed or fixed in this matrix can remain there for many days, unitl the horny cells are shed by the normal processes of cell renewal.

Chemicals with reservoir potential are likely to be water insoluble, with limited penetration capacity. The horny layer may be thought of as a trap for substances having these characteristics. In a sense, they dissolve in the horny layer and become an integral component of the membrane.

Vickers produced blanching (vasoconstriction) by occlusive application of a corticosteroid for 16 hours, after which the surface was cleaned off. The blanched area returned to normal skin color by the next day. To his surprise, merely reapplying the impermeable film over treated spots would reelicit the blanching. Indeed, vasoconstriction could be made to reappear

as long as 14 days after a single exposure! Evidently, the steroid was retained somewhere in the skin and could be mobilized by occlusion. Vickers proved beyond a doubt that the reservoir was in the horny layer. Blanching could not be made to reappear after intradermal injection of steroids or after stripping with scotch tape. Besides, radiotagged steroid was seen to be localized in the horny layer in radioautographs of skin.

A steroid reservoir was not established by open, application. The site had to be covered to fill up the reservoir. Moreover, it took at least 12 hours under occlusion to drive enough steroid into the horny layer.

By following the disappearance of radiolabelled corticosteroid with a surface gas-flow Geiger counter it was possible to detect fluocinolone acetonide for an average of 4 weeks! This is startling since the horny layer is completely replaced in about 2 weeks. One possibility is that there are receptors for the steroid in the living epidermis, the total turnover of which is about a month. Steroid receptors have since been demonstrated in the epidermis. With insoluble fluorescent dyes such as dansyl chloride, which bind avidly to horny but not epidermal cells, the disappearance time corresponds exactly to the renewal time, about 14 days, the period for the bottom-most tagged horny cell to make its transit through the entire horny layer (55). Dansyl is fixed and not mobilizable in a shifting pool within the reservoir.

Vickers found that the reservoir for salicylic acid lasted about 2 weeks, more in keeping with the kinetics of stratum corneum replacement. It should come as no surprise that radiolabelled penicillin was not detectable after a couple of days. This water soluble drug penetrates poorly and is meagerly sorbed.

An appreciable reservoir is attainable with a substantive agent like hexachlorophene, which readily partitions itself into the horny layer from certain vehicles.

Munro has shown that vehicles, which enhance penetration will establish horny layer reservoirs of penetrants (56). Thus, DMSO not only promotes

the diffusion of corticosteroids, estrogen and griseofulvin but establishes drug depots in isolated horny layers. Thus one could screen vehicles in a rather simple way by determining their capacity to establish a reservoir in small samples of stratum corneum. Scheuplein and Dugard have presented a more sophisticated version of this idea which is also more amenable to mathematical analysis (57). To penetrate, a substance must first dissolve in the horny layer. The latter can be looked upon as a solvent for the penetrant. That being the case, one can then determine the vehicle: horny layer partition coefficient in vitro. This has high predictive value for if the penetrant has high avidity for the vehicle, it will tend to stay in the vehicle. Otherwise, it will distribute itself towards the stratum corneum. This model has not been sufficiently exploited in optimizing vehicles.

The day is coming when these principles will be utilized to establish reservoirs for a variety of useful agents. In addition to drugs, one can immediately think of two applications that would provide high benefits, namely sunscreens and insect repellants. It would be a boon if these could be deposited in the horny layer so as to provide full protection for at least 24 hours.

## Animals vs. Human Skin

Serious efforts have been made to identify an animal whose integument resembles man's in respect to percutaneous absorption. This is an interesting scientific enterprise but I do not believe that we urgently need an animal model. The measurement of diffusion through human skin in vitro is accurate, relevant and reliable (see below).

Comparisons of absorption in man and animal in vivo have largely followed the design originally developed by Feldman and Maibach (58,59). This entails application of a radiolabelled chemical dissolved in ethanol to forearm skin at a small dose, 4 $\mu g/cm^2$. Urinary excretion is measured over the next few days and the percent of dose absorbed is calculated. It must be emphasized that this is an indirect measurement requiring application of a

correction factor derived from giving a similar dose intravenously. This attempts to take into account excretion by other routes or retention in body organs. When the calculated absorption is less than 5%, as with most corticosteroids, the chance of experimental error is great. The method is ingenious and useful, but crude. It must be noted that virtually all the absorption data on humans in vivo derives from Maibach et al using this model. Confirmation by distantly situated investigators is mostly lacking.

The thrust of all comparative in vivo studies is that absorption through the skin of small laboratory animals is consistently less than human skin. For example, absorption of haloprogen (an antifungal agent) exceeds 95% for rats and rabbits but is only about 10% in man (60). For cortisone, more than 25% was absorbed by rats and rabbits, compared to only about 4% in man. A feature worth emphasizing is the great importance of the penetrant itself as regards species differences. Thus, for poorly penetrating materials such as acetylcysteine (less than 5%), the difference between man and animals disappears. This is a foregone conclusion with crude methodology. Contrariwise, for easily absorbed molecules, the difference may either be negligible, viz, caffeine or very great, viz, testosterone (75% in rats and rabbits) compared to 15% in human skin.

Bartek et al found that absorption through the skin of miniature swine rather closely resembled that of human skin (60). This is very likely since pig skin has a well developed horny layer and the density of follicles is much less than in furred, murine species.

Maibach and his co-workers have touted the Rhesus monkey as a model whose permeability approximates man (61). While the results for hydrocortisone, benzoic acid and testosterone are comparable, the data base is insufficient to accept Rhesus as a valid model for man. I admit surprise, for while monkeys may be our relatives, this by itself hardly guarantees integumentary similarities. The monkey is not a naked ape. Indeed, pig skin is structurally closer to man's, though phylogenetically remote.

From the standpoint of feasibility and convenience, not to mention expense, one can hardly be overjoyed if compelled to use Rhesus monkeys and miniature swine.

In vitro studies comparing human to animal skin are more numerous and, I believe, more credible. Only a summary statement is appropriate here. The findings of different investigators show reasonable agreement, despite different methodologies, skin area, test compounds, etc. This subject has been put into perspective by Wester and Maibach (62). It is safe to say that the skin of common laboratory animals, rabbit, rat, mice, guinea pigs is appreciably more permeable than man's. Again, penetration through pig and rhesus monkey skin is closer to man. Still, there are disagreements which illustrate the complexity of the subject. Marzulli and his co-workers compared penetration rates in 11 animals, always finding mouse skin to have the greatest permeability (47). On the other hand, Stoughton thinks that human and hairless mice skin have comparable permeabilities (63). I dispute this. The horny layer of the hairless mouse is half the thickness of the human by my measurements. Stoughton's data are probably correct. However, he worked mainly with poorly penetrating materials; failure to find a great difference was almost to be anticipated. Thus, less than 5% of the various corticosteriods were absorbed in both species; there was even lesser absorption for tolnaftate. The results for thiabendazole (15% vs 10% in the mouse) are more in line with my expectations. Bronaugh's recent measurements support these cautionary views. In his in vitro comparisons, rat and human skin were about equally impermeable (64). The horny layer of rats is considerably thicker than hairless mice.

The inherent variability of the results has not received sufficient emphasis. Looking at the numbers, one finds that the variance is often great, with large ranges on both sides of the mean. The factor, which probably contributes most to this variability is unrecognized differences in the skin samples themselves. Rats and mice, even at birth, are not at all alike. We appreciate the great magnitude of individual differences in humans. In any event, a large variance can be dealt with only by large sample sizes.

In vitro vs In vivo Comparisons in Human Skin

Whatever the model, the measurements must be applicable to human skin. An issue of over-riding importance is the relevance of the in vitro model. I will state my bias forthwith. If diffusion is a passive process, then the penetration rates should be the same in living and dead skin, provided that circumstances are equivalent. This requires perfusion of the undersurface with a bathing fluid to mimic blood flow, thus keeping the concentration gradient maximal. Epidermis rather than whole skin is preferable to eliminate variables related to the large volume of dermis. In vivo, the rich superficial vascular plexus will remove a penetrant as soon as it passes through the epidermis. When skin is sectioned horizontally after applying radiolabelled drugs, the concentration in the deeper dermis is usually negligible owing to clearance by sub-epidermal venules in the papillary dermis.

There is no great difficulty in obtaining abdominal autopsy skin and preserving the isolated epidermis indefinitely. The advantages of the in vitro model are obvious. The question is: are the results relevant? I cannot imagine why they should not be. Again, the vitalists are quick to point out that living skin is a growing, metabolizing tissue, that sweats and secretes sebum, replacing its epidermis every month. These arguments are unpersuasive. Surface lipids play no role in percutaneous absorption (22, 53) and the subjects should not be sweating anyway. To be sure, dead skin does not desquamate. This cannot be important since only about one cell layer is shed per day and the in vitro measurements are usually completed in a day or so.

We have only the in vitro human data of Franz (65) to compare with the in vivo data of Maibach and colleagues. Measurements are given for only 12 compounds, not a very representative selection in my view, since it does not include some of the substances of greatest therapeutic interest, for example, corticosteroids, hormones, nitroglycerin, etc. The most notable feature of the two sets of data is the lack of agreement, with the exception, as one might expect, of compounds that penetrant very poorly, like hippuric acid, or very rapidly, like benzoic acid. For the rest, sometimes

there was appreciably more absorption in vivo then in vitro (caffeine and dinitrochlorobenzene) and sometimes the very reverse (nicotinamide, acetylsalicylic acid). Which to believe?

The prudent answer, at this unsettled stage, might be, neither. The discrepancies need to be restudied and the cause of the discordant results understood. Maibach's bias is all too apparent in his statement that "the in vitro method would not always be a reliable or accurate predictor of percutaneous absorption in living man (62)". That assertion would be equally reasonable if exactly reversed.

My prejudice is the very opposite. The in vitro data are more credible, on technical grounds alone. The methods are more precise, involve less experimental error and the variables are under much greater control.

If one honors theory, the in vitro and in vivo data ought to agree taking into account individual differences, correcting for body area, etc. When they do not, the in vivo data are suspect.

## Routes of Penetrations

### (1) The appendages

The integument is perforated by hair follicles into which sebaceous glands pour their lipid secretion. Additionally, eccrine sweat glands secrete a watery sweat through tiny pores which are visible only on the palms and soles. Below the surface these so-called appendages do not have a well-developed horny layer. Thus, they theoretically could serve as low resistance, rapid diffusion shunts, allowing easy ingress into the skin, by-passing the horny layer barrier. How importantly do they contribute to total flux?

This question has intrigued investigators, especially clinician researchers for a long time. They rather consistently endorse the idea of follicular shunts as an alternate route of entry. I think shunts are largely unimportant, a misperception, based again on histologic mirages. Rein applied dyes and found that the colored materials were concentrated around hair follicles.

He concluded that the dyes were leaking into the dermis from these channels (26). McKee et al were likewise impressed by the follicular route using histochemical markers to follow the passage of heavy metals, sulfanamides and dyes through skin (18). Shelley and Meltons clinical observations were entirely consistent (67). The first wheals which formed after topical application of histamine were small and always in relation to follicles. These gradually enlarged to become a confluent wheal, unmistakable evidence of preferential transfollicular absorption. Later, when radiotagged substances become available, the tendency for the label to concentrate around the appendages was again noted in radioautographs (68).

Nonetheless, the histologic appearances are susceptible to a quite different interpretation. The perifollicular localization may simply show where the markers tend to concentrate, perhaps because of an avidity for certain tissue components or because they are not readily removed. Such observations are purely qualitative and, especially because of the sensitivity of radioautographs, give no idea whether a little or a lot has penetrated.

Studies of absorption through different regions of skin have also seemed to suggest the importance of follicular shunts. For example, Wahlberg compared penetration of $^{22}$Na Cl through abdominal guinea pig skin, rich in follicles, with hairless skin behind the ears (69). The permeability through haired skin was 20% greater. In humans, Feldmann and Maibach found more impressive differences with labelled hydrocortisone in various body regions. Except for the scrotum with its flimsy horny layer, penetration on the head (face and scalp) was considerably greater than the forearm or trunk (70). This was mainly attributed to the high density of follicular shunts. This conclusion, which enjoys universal acceptance, is too simplistic. For example, Baker and myself found that tetrachlorosalicylanilide (a fluorescent dye) would diffuse more readily through facial horny layer than the trunk or extremities (71). The number of horny cell layers was also less on the face. Thus, its barrier is qualitatively and quantitatively inferior. The reasons usually given for the greater permeability of animal skin is the denser pelage with so many more shunts to convey chemicals in-

ward. I pointed out years ago that furred animals generally have a poorer horny layer (22). A thinner horny layer could account for greater permeability of animal skin. Finally, I rather suspect that lipid soluble chemicals which could diffuse down sebum-filled follicles would tend to stay in these channels. In this instance, sebum might be viewed as a vehicle for which the penetrant has high affinity, resulting in partitioning highly unfavorable for release into the skin. It is worth noting that Tregear tackled this issue years ago through direct measurement of tri-n-butyl phosphate through pig skin (72). He could find no evidence of preferential absorption through follicles!

The head region comprises less than 10% of the body surface. Almost everywhere else, the density of follicles is so low as to render this discussion quite academic. The follicles are far apart and rather small, bearing wispy, insignificant hairs. On the extremities, for example, the follicular openings cannot comprise more than 0.1 to 0.2% of the surface, an insignificant proportion.

As regards, the role of shunts one can do no better than to cite the reasoned arguments of Scheuplein who has viewed the problem in all its theoretical dimensions (73). He believes that for most molecules, steady state diffusion occurs directly across the epidermis and not through shunts. He allows that transappendageal absorption might be important in the early lag phase. For highly insoluble, poorly penetrating materials with low diffusion constants such as polar steroids, the appendageal route might become significant. This exception, I would add, would mainly apply to the face and scalp in humans. It is interesting that this is the very area where adverse local effects from corticosteroids are most frequent and serious (atrophy, telangiectasia, persistent erythema, etc.).

Paradoxical as it sounds, the "barrier" is the main avenue of penetration.

A few words are in order concerning sweat ducts as alternative pathways of diffusion. These, too, comprise a tiny fraction of the surface and

cannot play a significant role in total flux. Certain molecules can indeed enter the ducts readily and flow downwards with very little resistance. In vitro, the amount of aluminum chloride reaching the dermis is negligible. Yet, this metallic salt is an effective antiperspirant because it readily diffuses down sweat ducts creating casts which obstruct the flow of sweat (74). Water soluble electrolytes can freely enter water filled sweat channels and generate clinical problems such as heat rash (miliaria). Nonetheless, appendages can largely be disregarded as portals of entry. It would be easy to imagine that topical antibiotics such as clindamycin and erythromycin are effective in acne because they penetrate follicles, the very site of the disease process. The fact is they penetrate follicle-poor abdominal sufficiently well to establish bacteriostatic tissue levels in the dermis. I attribute the slowness of their therapeutic actions to their having to reach the dermis transcorneally from which they are "excreted" via follicles.

(2) Transcorneal Penetration

It would be remembered that the stratum corneum is not a homogenous structure; indeed, it is architecturally complex. As epidermal cells move upwards, the cell membranes become greatly thickened. At the level of the stratum granulosum just below the horny layer, specialized membrane-bound granules extrude their contents into the intercellular spaces (75). When markers as horse radish peroxidase are injected intradermally, they wind their way through intercellular spaces till they stop suddenly at this granular zone. Low resistance, diffusion channels between cells and here. Thus water soluble electron-dense substances like lanthanum selectively spread upwards through intercellular channels, stopping abruptly at the stratum granulosum. The heterogeneity of the horny layer has become even more clear through sophisticated techniques involving freeze feature, ultra-structural, and histochemical investigations (76,77).

Knowledge of the fine structure of the horny layer derives from studies with the electronmicroscope. The first impression a non-histologist gains from examining published pictures is that the horny layer is anatomically

variable. It takes on different appearances depending on who has prepared the specimens. As with light microscopy, processing can markedly alter and degrade the nature structure. A mischievous observer could fill up a whole volume of the Acta Artifacta with divertingly different images published by experts from around the world! We cannot engage here in this sport but the scene suggests caution in trying to imagine how molecules make their way across the horny layer. Some microscopists think that the horny layer has a stratified pattern. They describe zones made up of light and dark cells. These were originally called A and B cells by Brody and later characterized by Orfanos as osmiophilic and non-osmiophilic (78). In our experience, light and dark cells, swollen and compacted one, spongy and dense cells occur unpredictably at different depths. They are artifacts. A uniform appearance is hard to obtain, even in the same specimen. Other common artifacts include crevices and wide spaces. It is known that tight junctions do not occur in the horny layer. The cell membranes are not in contact with each other. The intercellular spaces are filled with multi-laminated structures which derive from membrane coating granules. The latter extrude their contents extracellularly just before the horny layer is formed.

Beautiful pictures of the lamellated material between horny cells have been published by Elias et al (79). These spaces have suddenly become the object of much interest through the systematic efforts of these same workers. A new controversy has been brewed.

The issue comes down to this. Do chemicals traversing the horny layer follow a winding, intercellular course or do they pass directly through the cells? Theoretical considerations led Blank and Scheuplein to the conclusion that intercellular pathways were highly improbable (12). Additionally, the volume of these spaces, amounting to perhaps 1% of the total, was far too small to account for the rates of penetration of various molecules. Elias and co-workers have now thrown down the gauntlet and challenge this view, basing their opinion on new biochemical and histologic insights (77). To them the intercellular route is an important one for lipophilic substances.

They think the barrier, at least to water, forms below the horny layer in the stratum granulosum. This is extremely doubtful since stripping eliminates the physiologic barrier but leaves the sub-corneal structures in place.

They present evidence from specimens fixed in butanol-osmium vapor that the intercellular spaces may be 3 to 7 times wider than previously thought, with dimensions of 80 to 100 nm. They discovered frequent bulbous dilatations adding to the potential intercellular volume. Nonetheless, the latter is still tiny in relation to the total space occupied by the horny cells themselves. The spaces are, of course, not empty being filled with a complex mixture which in part, serves to glue the cells together. Elias et al found that most of the lipid in the horny layer, comprising about 5%, is concentrated in the intercellular region. They were able to show that a fluorescent lipid-reactive reagent percolated through the stratum corneum via the intercellular route exclusively. They regard this as direct, positive proof of the intercellular pathway. However, we encountered this same kind of "proof" with light microscopy which showed localization of dyes around follicles, from which follicular pathways were speciously deduced. It is necessary to emphasize that the concentration of a dye or maker in a particular location may reflect nothing more than affinity of the marker for a special substrate. If the intercellular spaces are lipid-rich, as they probably are, then certain substances with high lipid affinity are likely to be selectively attracted there. No generalizations can be drawn from this kind of evidence.

As a matter of fact radioautographs with a variety of other chemicals, such as steroids, usually show diffuse penetration of the entire horny layer (53). Elias and his collaborators have been led to overturn conventional doctrine, emphasizing the determinant role of lipids in percutaneous absorption, concluding that the intercellular route is the dominant pathway of transport across the stratum corneum.

This thesis is novel, provocative and stimulating. Nonetheless, I believe their interpretation is wrong and goes well beyond the reported data.

This controversy will occupy students far more knowledgeable than myself for many years to come. This is not the place for gladiatorial combat.

If we take the most recent paper in this series as the climax of arguments against the "still prevalent physical-dogma", we can gain an appreciation of how far these investigators have departed from orthodoxy (81). Elias et al compared the flux of radiolabelled water and salicylic acid through abdominal and leg stratum corneum in vitro. This measurement was then correlated with thickness, cell layers and percentage lipid.

The findings are extraordinary, and, if true, require radical revision of standard teaching, a prospect the authors do not shy away from.

Firstly, they found that the two test agents penetrated more rapidly through leg skin in comparison to the abdomen. This is a startling result in view of the fact that leg stratum corneum was found to be thicker, with more cell layers. Ficks law would predict lower permeability for the leg.

Secondly, for each sample, whether leg or abdomen, there was no correlation between thickness, number of cell layers and flux. Theoretically, an inverse relationship should have been found. Instead, there was some evidence that flux increased with thickness, the very opposite.

Thirdly, there was an inverse relationship between percentage lipid and flux. The leg, being depleted in relation to the abdomen (mean 6.8% vs 2.9%) was more permeable. The authors use these data to attack traditional understanding of the principles governing percutaneous absorption. They are obliged to renounce Ficks law since penetration of water and salicylic acid representing water-soluble and lipid-soluble agents respectively was actually greater across the thicker stratum corneum. They suggest that "thickness may have little to do with stratum permeability", citing the relative ease with which certain substances penetrate the palms and soles. The latter, as I have stressed earlier, are qualitatively very different. They are horny pads to provide frictional and mechanical resistance and cannot be fairly compared to compact horny layers designed to be impervious.

Fick laws is a monument which will not be broken by Elias et als curious findings. It has been thoroughly substantiated by many workers using a variety of membranes. If the data cannot be reconciled with Ficks law, then I make bold to say that the data are wrong. I certainly do not believe that the leg is more permeable than the abdomen. In our indirect measurements of permeability base on whealing and blistering responses, we find a clear cut inverse relationship between the number of horny cell layers and penetration, in concordance with expectations. It takes longer to blister leg skin with ammonium hydroxide in comparison with skin at the trunk (48). Moreover, DMSO wheals are less easily elicited on the legs (82). We invariably find this to be the case for a variety of excitants. Leg stratum corneum is definitely thicker and has more cell layers. These very features account for its lesser permeability.

To Elias et al lipid content, is much more significant than stratum corneum thickness and number of cell layers. They predict that regions of high permeability will be found to have a low percentage of lipids, even if the composition is the same. I do not accept their statement based on histochemical evidence that there are no lipids within horny cells and hence diffusion of lipophilic substances directly through horny cells is improbable. I prophesize that future studies will show that direct penetration through horny cells is the dominant pathway by far.

Miscellanea:

Vehicles have a profound effect on penetration and much pharmaceutical effort is directed toward optimizing them. This process is also becoming less empirical. Trial and error is giving way to theoretical predictions. Katz and Poulsen have reviewed this subject thoroughly without losing sight of practicalities (83).

Penetration of course increases with increasing temperature. This is of greater theoretical than practical interest. Under most circumstances, surface temperatures vary within a fairly narrow range. Through various

devices, including clothing, heating, cooling, man take steps to avoid the discomforts imposed by meteorologic externalities. On the other hand, elevated temperatures do more than promote diffusion across the horny layer, an effect which is by and large small. They increase blood flow and alter metabolic processes. Thus, the effects of temperature are quite complex.

Finally, I do not think we can dismiss blood flow as easily as Scheuplein suggests (13). True enough, the micro-vasculature of human skin is so richly developed that the concentration of penetrants within capillaries and venules is practically zero. It is argued that the concentration gradient is maximal since the diffusing molecules cannot accumulate in the internal side of the horny layer. Thus, changes in blood flow could hardly influence flux under most normal circumstances.

Our observations, crude as they are, do not accord with this doctrinaire approach. I think the extra-vascular concentration in the superficial portion of the dermis may be significantly influenced by blood flow. I do not know how to otherwise account for the marked seasonal differences in the skins reactions to various physiologic agents and to chemical irritants. Lehmann in our laboratory has convincingly demonstrated decreased reactivity in the summer months as compared to winter. I assume brisker blood flow in accommodated subjects in the summer time. Circulatory effects deserve more serious study.

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