REVIEW ARTICLE

THE RELEASE OF MEDICINAL SUBSTANCES FROM TOPICAL APPLICATIONS AND THEIR PASSAGE THROUGH THE SKIN

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When medicinal substances in the form of topical applications like ointments and creams are applied to the skin the effect which is desired may be either local or systemic. Therefore a knowledge of the rate and extent of release of the medicament from the vehicle in which it has been incorporated is important. The large number of vehicles and bases now in use for the presentation of medicinal substances as skin preparations underlines the need for suitable methods which estimate the rate and extent of release of medicaments. There is a need, too, for precise methods for evaluating the amount and extent of penetration and systemic absorption of medicaments from their vehicles.

The Skin as a Barrier

The properties and structure of the skin influence the extent of penetration and absorption of a medicament applied to its surface. The skin consists of two layers, the outer epidermis and the inner dermis. The outer epidermis is composed of stratified epithelium rich in lipids and esters of cholesterol. The superficial layer is horny, tough, and keratinised, and also presents a greasy surface which prevents or delays the penetration of water and aqueous solutions. The skin is electrically polarised and behaves like a membrane with a negative charge on the outside. It is cation permeable and anion impermeable, and this is one explanation of the general impermeability of the skin to electrolytes, although substances in aqueous solution can be introduced by electrophoresis. But no systemic absorption results from penetration into the outer epidermis since it is avascular.

The inner dermis is composed of a meshwork of fibrous and elastic tissue which is well supplied with blood vessels and merges into, and is continuous with, the underlying fat. The control of the flow of blood by the variation of tone of these blood vessels regulates surface temperature; also they serve as the nutritional vessels of the dermis and should a substance penetrate the outer epithelium it may be rapidly absorbed in the capillary bed.

The greater part of percutaneous penetration takes place by way of the appendages of the skin—hair follicles and sebaceous and sweat glands—so that before penetration may take place, there is yet a further barrier to systemic absorption; this is the sebum, a secretion of glycerides and fatty acids.

The presentation of medicaments in ointments, creams or lotions may be considered from three aspects. Firstly, the liberation of the medicament from the base or vehicle; secondly, the penetration of the outer epidermis of the skin by the medicament; and thirdly, the absorption of the medicament

into the bloodstream. Among the factors influencing penetration and absorption through the intact skin are the mode of application, the vehicle, the various physico-chemical properties of the medicament, the base, dermal secretions such as sweat, sebum, tissue lipids, and the extracellular fluid.

Penetration readily occurs through broken skin because the protective barrier of outer layers has been removed. Perhaps unbroken skin is a rarer state than we imagine; the modern concept of hygiene demands, in varying degree, the removal of sebum with detergents, and the abrasion of the skin with various foreign tissues.

The Problem Defined

While the purpose for which a topical application is intended should be considered in the design of any test for the evaluation of its efficiency, the number of variables in the intended system is often such that to obtain any result it is more advantageous to investigate one aspect of the system—usually the rate of release. This has given rise to numerous *in vitro* techniques. These tests are of value where the medicament is required for local effect only, as with antiseptic ointments. When penetration and absorption of the medicament into the skin is important, histological and histochemical results are needed to measure the rate of release. The rate and degree of absorption of a medicament which gives rise to systemic effects can be assessed by clinical effects, or tests on animals in which the concentration in blood, urine, faeces or tissues is measured. Pathogenic skin conditions are numerous and preclude generalisations so most *in vivo* assessments are made on healthy intact skin to avoid increasing the number of variable factors in the system. This approach itself is open to criticism in that the true state of affairs is not extant but if the approach is to be rationalised, work must move to the specialised from the generalised rather than plunging directly into an assortment of individual skin conditions.

Recent reviews[1–13] have discussed the absorption of drugs, and it is proposed to limit this review to a survey and evaluation of the variety of methods which have been devised to estimate the rate of release, amount of penetration and absorption of the applied medicament from the vehicle. Chemical, physical, pharmacological, toxicological, histological, and microbiological methods have been adopted. In this range of tests and techniques, some are concerned with liberation, some with penetration and some with absorption. To rationalise the range of methods a classification has been attempted.

In Vitro Methods

Diffusion without a membrane

(i) Chemical and Physical
(ii) Microbiological

Diffusion with a membrane

(i) Chemical and Physical
(ii) Microbiological

In Vivo Methods

(i) Blood, urine, faeces analysis
(ii) Tissue analysis
(iii) Characteristic Reactions
(iv) Histological methods
(v) Clinical methods

Radio-active Tracer Methods

In Vitro Methods

In vitro methods are of limited value but they are a means of assessing the ability of a vehicle or base to liberate medicament under the conditions of the test. Those reported are of a comparative nature, and most are empirical which prevents results from being compared with those from other techniques. Lockie and Sprowls[14] hold that the study of ointment bases by *in vitro* methods is essentially a study of diffusion rates, neglecting the importance of the base as an emollient or protective. The methods seem to fall into two categories of diffusion methods that use a membrane and those that do not. In both of these categories chemical, physical and microbiological estimations have been devised.

Diffusion Methods Without a Membrane

The assumption made in most of these tests is that the distribution of medicament between the vehicle or base and the area under treatment will be similar to the distribution between vehicle and medium of the test, although this is doubtful, particularly when one considers the complexity of skin fats with the simple media—water and saline—often used. The distribution will be a function of the partition coefficient, assuming the medicament is soluble in both phases; it will also be governed by the rate of diffusion of the medicament from within the base to the surface. A base in which the medicament is not soluble but dispersed, can make available only such material as can be leached from the surface layer by the extraction medium, unless this itself is soluble in the base. This factor seems to have been disregarded in some observations; it is, of course, the same factor which accounts for the very small quantities of medicament released particularly from mineral oil bases. With such base and medicament relations a badly made preparation presenting gross particles of medicament to the extraction medium will give anomalous results.

Chemical and Physical Estimations

Demonstrating the principle simply, Seelman[15] showed diffusion of salicylic acid from a lard base by covering the ointment with a solution of ferric chloride, when the colour diffused quickly throughout the supernatant; soft paraffin did not release the acid to give a colour.

Hawking[16] followed the course of the topical release of sulphonamides with a static method of measuring diffusion by estimating the amount of sulphonamide, from a dispersion, diffusing into a known surface area of agar or gelatin incorporating Erlich's reagent (an acid solution of *p*-dimethylaminobenzaldehyde). The sulphonamide reacts to give a yellow colour the depth of which was measured and an analysis of the results made. Waud and Ramsay[17] also used a static method, similar in technique, to measure the diffusion of sulphonamides from hydrophilic and non-hydrophilic bases with and without sodium lauryl sulphate. Lockie and Sprowls[14] sought a mathematical basis using the rate of diffusion of sulphonamides from bases into an agar gel incorporating Erlich's reagent. Hawking[16] also used a cellulose film cylinder into which gelatin was

poured and then 5 ml. of a suspension of sulphonamide added. The use of the cellulose container made possible the slicing, transversely, of the gelatin and, after standing the uncut cylinder and contents at room temperature, 3 mm. slices were cut and analysed for sulphonamide by a colorimetric method.

A colorimetric method was used by Bandelin and Kemp[18] to assay sulphonamide release. The sulphonamide ointments were applied evenly around the inside of standard test-tubes to which either saline or serum was added, the tubes were incubated at 37° for varying times after which the fluid was decanted and assayed colorimetrically. Surface-active agents markedly increased the rate of release of drug.

Howard[19] also used the colorimetric method to estimate the release of medicament from ointment spread on a watch glass into surrounding water at 37°. A criticism of these methods is that by allowing only static diffusion the rate of release would be less than would occur where the extraction fluid was in motion as with blood and serum. To overcome the disadvantages of Hawking's method, Fuller, Hawking and Partridge[20] estimated the rate of diffusion of sulphonamides from a glycerol-gelatin jelly into a stream of water moving at 20 ml./hour. With sulphanilamide, which is about 10 times more soluble in water than most other sulphonamides (except sulphacetamide), diffusion was rapid until the surface layer of the jelly was exhausted. After 25 hours, conditions approached equilibrium, probably as the result of diffusion from the inner layers of the jelly. A steady rate of diffusion was obtained with the less soluble sulphonamides. The method is simple and elegant, and although water and not saline or tissue fluid was used in the extraction, the authors applied the sulphonamide to a standard wound, exposing subcutaneous tissues in rabbits, and claim a satisfactory agreement between their *in vitro* and *in vivo* results. But this *in vivo* method is not, of course, a measure of absorption through intact skin.

Microbiological Assay Methods

Since topical applications are often intended to be antiseptic a number of methods and techniques have been devised which depend upon a form of microbiological assay. The familiar arguments for and against the methods of assessing disinfectants would equally apply here. The tests aim at assessing the antiseptic value and also give an indication of the rate and degree of release of the medicament from the vehicle, but, of course, different organisms and bases are used and comparisons should be made only when all the conditions of bioassay are met, which seldom occurs.

One of the earliest methods was devised in 1895[21] when glass coverslips were treated with a broth of *Serratia marcescens*, dried and then introduced into the ointment for a fixed period of time. The slips were then washed with ether, transferred to sterile broth and incubated. The effect of the ether on the organisms does not appear to have been investigated. Cold cream and lanolin were found to be the most effective bases for the release of the antiseptics used. Cheyne[22] spread antiseptic ointments on coverslips which were then laid under agar in a petri dish. After inoculating

the surface of the agar with *M. pyogenes var. aureus* and incubating, zones of inhibition were observed; lanolin was the most and the hydrocarbons the least efficient in releasing phenolic antiseptics. As these are far more efficient antiseptics in aqueous media, this result could be explained by the fact that cold cream and lanolin make phenol available to aqueous surroundings more easily than mineral oils by virtue of their own water content or their ability to incorporate water and thus give a greater zone of inhibition.

Reddish[23] devised the most widely used test for assessing the effectiveness of antiseptics included in ointment bases, and this with certain modifications was eventually adopted by the United States Food and Drug Administration[24] and remains the only official test in being. In the original, plates were inoculated with a culture of *M. pyogenes var. aureus* and spread with the test ointment melted at 37°, controls using base without medicament also being used. After incubation the width of the zone of inhibition was taken by Reddish to be an indication of antiseptic value. His suggestion that the nutrient agar of the petri dish simulated the conditions met with in wounds and skin since "it is permeable, semisolid, isotonic and constitutes a valuable laboratory means of approximating the conditions found in human and animal tissue" is open to question, as apart from physico-chemical differences there is no cell membrane to penetrate, no keratin, cell detritus, pus, skin flora, excretions or appendages, all of which are known to influence activity and absorption. The method is also limited to antiseptic preparations and is not applicable to preparations which have no antibacterial property.

Reddish and Wales[25] and other workers[14,26] using this and similar tests have shown the much greater efficiency of emulsified bases over fat, oil, and wax bases as antiseptic carriers. Reddish's test was modified by the use of weighed quantities of ointment spread over a definite area, and it was shown that antiseptic efficiency was diminished or entirely absent with fat, oil, or wax bases, while oil-in-water emulsion bases gave a greatly increased antibacterial value[27].

Pillsbury, Livingood and Nichols[28] proposed a technique in which the hands were scrubbed in a standard way and the number of organisms removed was estimated by colony count of incubated rinsings. Ointment was rubbed on each hand and forearm, allowed to remain for a stated time, removed and, after washing again, the number of organisms remaining estimated, comparison of diminution in count being the criterion of efficiency. The objections to this method are numerous; the main criticism is that it introduces too many variables to be of practical value as a reliable technique.

Diffusion Methods using Membranes

The use of a membrane is an attempt to simulate *in vitro* the barrier which is presented by the skin to a topical application. Both artificial and natural membranes have been used, and while the assumption is made that the process of penetration in the skin is similar to the quantitative diffusion through a membrane, this does not make any allowance for

differences in physico-chemical properties of "dead" membranes and living tissue, the latter presenting a much more complex system both physically and chemically.

Chemical and Physical Estimations

In an attempt to make a simple evaluation, Rae[29] incorporated sodium chloride into various ointment bases which were then introduced individually into glass tubing to one end of which was attached a cellulose film membrane. The ointment was gently forced to the membrane end of the tube and this was then immersed in distilled water for 24 hours. The chloride diffusing out was assayed with silver nitrate. Rae states that "the various results obtained probably represent what takes place when the ointment is applied to broken skin"—a quite uncritical statement in view of the method, "medicament" and bases employed. It is hardly surprising that the greatest release was from a 5 per cent pectin jelly. No temperature was stated, an artificial membrane and a completely ionised salt were used, and no allowances were made for the effect of electrolytes in blood or serum.

In 1891, Luff[30] immersed, in water at 37°, sheep's bladders in which were suspended ointments of paraffin, lard and lanolin base with potassium iodide, phenol and rescorcinol as medicaments. He found paraffin showed the quickest and lanolin the slowest rate of release, a result at variance with later workers.

Coran and Huyck[31] used a method suggested by Izgu and Lee[32] in which diffusion from ointment in a hollow cylinder, placed in the centre of a filter paper moistened with indicator solution, was estimated by measuring the distance to the outer edge of the indicated ring on the paper. Izgu and Lee used salicylates and ferric chloride impregnated paper; Coran and Huyck employed sulphonamides and Erlich's reagent, and assessed their results in similar manner to that adopted by Lockie and Sprowls and compared the results of these authors with their own.

Microbiological Assay

The rate of release of penicillin[33] and sulphonamides [34] was estimated from various bases placed in cellulose film bags immersed in saline at 37° for a fixed time. The saline was estimated for activity by the F.D.A. cup-plate method. Clarke and Davies[35], in an interesting modification of the cup-plate method, poured agar plates which were dried for 2 hours, after which 1 ml. of a 1 : 10 dilution of a 24 hour culture of *M. pyogenes var aureus* was added. Four sterile 1 inch squares of cellulose film were then placed on each plate, and after 45 minutes incubation the films were carefully spread with the preparations under test and the whole incubated overnight, the plates were then examined for zones of inhibition. With phenyl mercuric nitrate and proflavine sulphate an increasing rate of release was observable from fatty base, water in oil base, oil in water base to jelly. Velu and colleagues[36] examined the diffusibility of antibiotics from various ointments and creams by placing them in dialysing tubes and assaying samples at intervals.

In Vivo METHODS

Most absorption through the intact skin occurs by way of the appendages. Increases in rates of absorption are usually seen with the use of solvents or bases that remove the protective layer of sebum and skin fats and thus aid penetration; absorption is also increased when the medicament is itself absorbable. The steroids, salicylic and boric acid are good examples. Numerous methods with animals have been devised to estimate both penetration and absorption of medicament; generally the results of animal experiments may be extended to man.

Blood, Urine and Faeces Analysis

After its cutaneous application, the detection of a substance in the bloodstream, urine, or faeces offers the most conclusive proof of its absorption but care must be exercised in accepting these figures as a quantitative estimate. Blood levels have been accepted as a measure of the absorption of topically applied sulphonamides[37–39]. Woodward and others[40] determined sulphathiazole levels in blood samples and in catheterised urine samples from rabbits after the application of an ointment to a measured area of clipped skin. Bases containing propylene glycol were found to be superior to fat or oil bases when using the absorption of sulphathiazole as a criterion. The addition of certain surface-active agents improved absorption. A comparison of ointment bases using rats by Meyer and colleagues[41] employed phenolsulphophthalein and potassium iodide as chemical tracers. The time taken for the first colouration of the urine in sodium hydroxide with phenolsulphophthalein was noted, while for potassium iodide a chemical analysis was made. These results and those obtained with the same bases from the agar plate method were correlated. Lund[42] studied the absorption of calcium and sodium penicillins from ointment bases by assaying the drug in urine samples.

That mercury is absorbed percutaneously there is no doubt: the mechanism is presumed to be by combination of the mercury with the sebaceous fatty acids to produce oil-soluble salts. The rate of absorption is modified by the choice of base. Mercury is conveniently estimated in the body fluids, tissues and excreta by routine chemical analysis[43].

Tissue Analysis

If selective absorption of medicament occurs in particular organs or tissues misleading information will be obtained by estimations of blood, urine or faeces levels. Laug and his colleagues[44] found one hundred times as much mercury in the kidney as in the rest of the body and urine after the application of mercury ointments. Differences in the efficiencies of the ointments were observed. Lang and Kunze also estimated the absorption of lead by the kidney, liver, muscle and lung from different vehicles applied to rats[45].

Care must also be exercised in accepting systemic blood levels as an estimate of the amount of absorption. Since the blood level represents drug in transfer it accurately reflects several unknown transfer processes. The first is the rate of absorption from the skin site; the second is the

storage in depots like fat (hexobarbitone) or heart and kidney (mercury); the third is the rate of excretion in bile and urine. Frequently, as in the classical instance of sulphonamides, the kidney is functioning both as an organ of excretion by glomerular filtration and of conservation by tubular resorption. This latter mechanism is common to many drugs[46].

An ingenious method of measuring release and penetration of drugs devised by Hunter and Smith[47] uses chick embryos. The authors injected into the natural air sac of fertilised eggs ointment bases containing antibiotics. The chorio-allantoic membrane served as the medium for penetration, toxicity was determined by the number of embryos remaining alive after varying times. An assessment of release of the antibiotics was made by assaying aliquot portions of the allantoic fluid. The authors justify their method by a number of arguments. The method employs a living membrane devoid of glandular ducts or hair follicules which eliminates absorption through such appendages. The living tissue is covered by a relatively dry non-living membrane which is thin and therefore allows rapid analysis, and this give a biological system from which small samples can be assayed *in vivo* without excretion problems. The technique is simple and the method cheap. However, the lack of keratin in the non-living shell membrane and chorio-allantoic membrane and the use of embryonic tissue with remote phylogenetic relation to mammals constitute salient criticisms of this method giving statistics of doubtful application to man.

Characteristic Reactions

Pharmacological or physiological end points have been adopted as measure of the time taken for the passage of a drug through the skin to produce a systemic effect. Macht[48] used strychnine to measure the action of various ointment bases on the course of penetration of alkaloids. Convulsions were taken as the end point in tests on rats and mice. Macht concluded that the use of ointments did little to improve and may even have hindered penetration. Walzer[49] demonstrated the percutaneous absorption of an antigen by first sensitizing passively a skin site to the antigen and then proving absorption by the production of an urticarial reaction at another site 24 to 48 hours later.

The effect of the passage of hormones through the skin has been the subject of wide research; a few of the many references will be quoted. In 1929 Zondek[50] demonstrated the physiological effects resulting from the passage of sex hormones through the skin. Moore and others[51] discussing the effects of androgens and oestrogens on the skin of guinea pigs stated that "there is yet a lack of appreciation of the readiness with which substances are taken up by the skin and are effective in the body". Nelson, Greene and Wills[52] were critical of this work as the number of animals in the experimental groups used by these workers was small and the data not statistically analysed. This latter criticism can be applied to much of the work on percutaneous absorption, and was one of the criticisms of the Hadgraft, Somers and Williams[53] paper presented at the 1956 British Pharmaceutical Conference, although in an earlier paper Hadgraft and Somers[54] attempted a statistical examination but made the point that

different bases were used which influenced rate and magnitude of release, and with test and standard not having the same contribution the principles of bioassay were confounded. They concluded in this work that a very large number of animals would be needed to obtain a high degree of precision. Smith, commenting on the paper, considered the assay valid because the *same* medicament was being compared in different bases.

The method presented by Hadgraft and Somers to the British Pharmaceutical Conference in 1954 was an interesting application of characteristic effects. Eserine potentiates the effect of acetylcholine which induces the secretion of opaque reddish-brown tears in the rat. The acetylcholine 50 μg. was injected subcutaneously, then the rats were inuncted on a shaved area on the back with bases containing eserine. The response was given an arbitrary classification of numbers based on the amount and colour of secretion. The drawback of this method was the difficulty of carrying out this procedure on a number of animals large enough to give reliable results, and the authors contended that calculation of a statistic was not in accordance with bioassay principles, paraffin used as a standard being compared with other bases. From their results it was concluded that the absorption of eserine was better from lard, arachis and castor oil than from white soft paraffin. Only oily or fatty bases were used. It would have been interesting to see the results of release of eserine from oil-in-water emulsion bases, ethanol or ether in which the solubility is different.

Zondek[55], making use of ethanol (96 per cent), ether and benzene as vehicles for the application of oestrogen was able to detect only little difference between the cutaneous and subcutaneous administration of the drug necessary to produce oestrus in castrated mice. Nelson and his colleagues[52] have shown that percutaneous administration of testosterone was more effective in ethanol than in oil as measured by the increase in weight of the prostate and seminal vesicles of rats. Here two factors were probably extant, the defatting action of ethanol and a partition coefficient more favourable to release of the steroid from this solvent than from oil.

Heparin creams have been tested by German workers who noted a significant increase in the coagulation time of the blood of the rabbits used[56].

Another interesting application of a characteristic reaction has been used to measure the effectiveness of ointments containing local anaesthetics. Using a modified Harding-Wolff-Goodell pain threshold apparatus, Lucas and Guth[57] measured the response to the stimulus of radiant heat on the blackened tails of rats to which were applied Simple Ointment, Hydrophilic Ointment and Bentonite Ointment U.S.P. XIV with the anaesthetics incorporated. The base alone was used as control. With this pain threshold method, Brockmeyer and Guth[58] showed that bentonite was an efficient base for the release of cyclomethycaine. These results are subject to the obvious criticism that heat has been used and absorption no doubt facilitated by the increased peripheral blood supply which will result. Heat will also reduce the viscosity of the bases which will assist release. The comparison is legitimate but may prove unreal.

The protective effect of topically applied chemotherapeutic agents has been used to demonstrate absorption and could be used to evaluate vehicles. Zondek[59] studied the application of external disinfectants, in particular *p*-chloroxylenol, in protecting rats infected with streptococci and pneumococci, and this protective effect has been utilised in clinical trials. Green[60] investigated the release of sulphonamides from creams by two methods using mice. Neither involves skin penetration, and therefore only measures rate of release of medicament from depots within the body. In the first method, groups of 5 mice were injected with 0·1 ml. of the cream intraperitoneally and blood levels estimated for 6 hours after injection. The other method was to measure the survival times of mice, infected with *Str. pyogenes* in the thigh muscle after subcutaneous injections of the creams. Infected untreated animals were used as controls.

Histological and Histochemical Methods

A variety of procedures under this heading has been adopted to evaluate drug release from vehicles. Biopsies were made at intervals by Strakosch[61] to assess the time to produce keratolysis by sulphur, salicylic acid, rescorcinol, and other medicament-containing bases.

Duemling[62] adopted a method similar to that of Eller and Wolff[63] in which a study was made of permeability and absorbability of preparations into the skin. Shaved rabbits were treated with test materials on one side of the back, the other side being used as control. Skin biopsy showed much more rapid penetration and a greater depth was obtained when fatty substances were applied with wetting agents.

The penetration of sulphathiazole through the intact skin of rats, rabbits, and men, was measured by means of tissue analysis, and the results obtained from the application of wet dressings, iontophoresis and ointments have been compared[64]. Penetration was examined from water-in-oil and oil-in-water emulsions of sulphanilamide; increase in concentration of sulphanilamide failed to increase the degree of penetration. The use of solubilisers and wetting agents did not give improved results, and injured skin took up greater amounts of sulphanilamide than intact skin. Of the sulphonamides tested, the very soluble sodium sulphacetamide had the greatest tissue concentration[65,66].

Dyes[67–71] and fluorescent materials[67,68,72] have been used to demonstrate skin penetration. Harry[67] examined histologically human post-mortem material, and rabbits, rats and guinea pigs after application of fats, oils, emulsions and aqueous solutions containing dyes and active chemical agents. He concluded that oil, fats and aqueous preparations in general do not penetrate the intact epidermis to any appreciable extent, but that lipids such as cholesterol and lecithin could increase the penetrating property of liquid paraffin, and the presence of polar groups conferred penetrant properties upon mineral oils and greases.

Clinical Methods

Clinical methods have amply demonstrated the systemic absorption of medicaments, but many of the methods do not effect an evaluation of

drug release. In 1911, Wild[73] used an "analysis by difference" technique which he continued with Roberts[74]. The method is primitive, and the results should be viewed with caution. A known weight of ointment was inuncted on the arm for a given time, the excess ointment was then removed by a tared razor blade, and the weight difference was the quantity absorbed.

Moncorps[75,76] carried out the first comparative clinical trials when he measured the amount of salicylic acid eliminated after its cutaneous application in various bases. The absorption of salicylates and iodides has been examined extensively. Brown and Scott[77] studied the absorption of salicylates from various vehicles using the hands as the area of application. The excretion of salicylate in the urine was assayed. They observed a trend to increasing absorption coinciding with a downward trend of partition coefficients. In a general discussion on cutaneous absorption they point out that it has generally been considered that a substance must possess both oil and aqueous solubility to penetrate the human skin, and that an extremely high or low partition coefficient is less conducive to absorption than intermediate values. Zondek[59] showed that the blood of patients who had received percutaneous treatment with *p*-chloroxylenol was able to inhibit the growth of staphylococci and that an application of this medicament brought about disinfection of the urinary tract because of its presence in the urine.

Using phenolsulphophthalein excretion, after application to the skin, and its colorimetric estimation in the urine, it was demonstrated by Nadkarni and others[78] that a vehicle containing propylene glycols promoted greater absorption than an oil or paraffin base. Propylene glycol and surface-active agents have been shown to aid the passage through the skin of acetylcholine chloride, pilocarpine nitrate, atropine sulphate, hyoscine, ephedrine and histamine phosphate, the substances themselves being used as pharmacological indicators[79]. Greatest absorption was shown to have occurred in the most hairy areas of the body, while no evidence of absorption was noted when the drugs incorporated in penetrating types of vehicles were applied to the palms of the hands and the soles of the feet, areas where hair follicles are normally absent. Here the keratinous layer is thicker than elsewhere on the body, but the results support the contention that absorption occurs only via the appendages.

Jacobi and Lantzsch[80] proposed a photometric method of estimating the penetration of ointments and creams after 10 drops of a 1 per cent solution of Sudan Red 5B in mineral oil had been admixed with them. 0·1 g. of ointment was used on a circle of the skin of 4 cm. in diameter on the inside of the underarm. Filter paper squares measuring 25 cm.^{2} were laid upon the treated skin for 30 seconds by means of the cuff of a blood pressure apparatus. This procedure was repeated with fresh paper until no more colour could be removed from the skin. Untreated skin, treated skin before and after removal of the surplus ointment, and an arbitrary standard (barium sulphate) were given a photometric value, and from these a value for the absorption or penetration of the ointments tested was calculated.

Radioactive Tracer Methods

The application of tracers to the study of permeability in biological problems arose from the pioneer work of Hevesy. Isotopic techniques have now been introduced to evaluate drug release from topical applications and probably offer the most accurate *in vivo* means of assessment, and they are of particular value where the biochemical processes following absorption are being investigated.

Where the information which is obtained is of the same nature as that from chemical analysis, tracer techniques should not necessarily displace accepted analytical or quantitative procedures and in general they should only be used where results will be more accurate than those obtained by other methods. Up to the present time only relatively simple compounds have been used for the investigation of absorption through the skin: perhaps with a reduction in cost and greater availability of labelled compounds, their penetrant and medicinal properties will be increasingly examined in animals. It is doubtful, with the evidence of cumulative effects, that further work on man will be permitted.

Barker, Christian and De Kay[81] modified the agar cup plate method to estimate the release of ^{131}I incorporated as salts in hydrophilic bases. The agar was not seeded with a test organism but weighed amounts of ointment were placed in each plate, and after incubation strips of agar were removed and the counts per minute determined. In all cases the iodine diffused throughout the medium. Agreement with the cup plate method was found in all but U.S.P. XIV hydrophilic ointment, where the zone of inhibition was the smallest with the official method while by the radioactive tracer method the release of iodine was the greatest.

Johnston and Lee[82] using sodium chloride activated by deutron bombardment in a cyclotron tested absorption from fatty bases. The salt was dissolved in water, incorporated into the bases and a weighed portion tested for activity. One gram was inuncted into the upper arm of young males and the counts per minute from the left hand measured. Urine samples were also taken. Anhydrous lanolin gave the best results.

Czetsch-Lindenwald[83] in his experiments was unable to demonstrate the absorption of ointment bases containing deuterium oxide as a tracer, and it was suggested that only penetration had taken place although Szczesniak and others[84] were able to demonstrate the penetration of deuterium oxide through the skin of rats by measuring the content in the blood.

Cyr and others[85] studied the absorption of sodium iodide, labelled with ^{131}I, from lard, woolfat and soft paraffin when applied to albino rats. The ^{131}I was determined in the thyroid, after killing the animals; the amounts passing through the skin were small, a result also observed by Hadgraft and colleagues[53]. Skauen and others[86] used a similar technique and found hydrous wool fat to be more efficient in releasing ^{131}I than lard.

The permeability of frog skin has been examined extensively by means of tracer techniques. The ability of frog skin to allow the passage of both liquids and gases is well known, but as this ability decreases as the phylogenic series is ascended the results obtained by this method are hardly

applicable to man. Nevertheless, an evaluation of the effectiveness of antiperspirant preparations using isolated frog membrane and radioactive iodine as a tracer showed that astringents increased the rate of iodine ion penetration[87,88].

Other isotopes have been used in tracer work on absorption. For example, Loeffer and Thomas[89] used radioactive strontium in the form of the chloride for the determination of absorption through rat skin, both intact and broken, and the use of radioactive zinc in the form of the sulphate has shown that absorption was least from fat bases but was increased by the addition of soap[90].

Edwards[91] has studied the absorption of topically applied amino acids in guinea pigs using methionine labelled with ^{35}S. The presence of ^{35}S in cystine in newly grown hair was demonstrated.

Hadgraft, Somers and Williams[53] using diiodofluorescein-^{131}I have examined percutaneous absorption in rats from five different bases containing the tracer. The radioactivity of the blood was assessed, and only very small quantities of radioactivity were detectable. The results suggest that absorption was better from hydrous ointment and cetomacrogol than from lard, white soft paraffin and hydrous emulsifying ointment. The authors suggest that this would be expected by the theory that the skin surface is repellant to aqueous solutions, and when the external phase is oily, as in hydrous ointment, or the base has both lipo- and hydro-philic properties as cetomacrogol, miscibility with the sebum is facilitated, which allows the medicament to come into contact with the absorbing cells at the base of the follicles.

Clinically Lange and Evans[92] applied an ointment of lanolin containing radon to patients, and the amount of radon in the expired air was taken as a measure of the amount passing into the bloodstream. The application of thorium X to human skin has demonstrated that penetration is by way of the appendages[93]. This confirmed work by Witten and others[94,95] who examined biopsies of tissue which had been treated with thorium X in various vehicles.

Present Status of the Problem

This survey reveals that while a certain elegance of technique has been evolved, a fundamental basis is still lacking upon which experimental work, to be of real value, can be placed.

In vitro methods begin at a disadvantage by being *in vitro* while the variable reception encountered in different animal species can also make for misleading results *in vivo*. Few authors have set out to make any but an arbitrary assessment based upon their own terms. The results are usually not comparable with other methods.

It is possible that an explanation in terms of Brodie's[46] extrapolation of early theories might offer a basis for experiment. Many of the reports reveal that lipid-soluble drugs, provided they are made available by a vehicle allowing diffusion, can pass rapidly into the body after application to the intact skin. Harry[67] in 1941 commented "It is a remarkable fact that nearly every substance at present known to be absorbed is oil-soluble

(salicylic acid, phenol, rescorcinol, β-naphthol, iodine, vitamins A and D, while mercury can combine with fatty acids to produce oil-soluble salts)". These examples and those of steroids and recently the organo-phosphorus insecticides suggest that in alignment with these ideas some physico-chemical factor common to all should be considered. Brown and Scott observed that in the absorption of boric acid by the skin "a highly specific property of boric acid appears to be involved". We have already noted that the skin behaves like a membrane with a negative change on the outside. This means that electrolytes are not likely to be permeable and that anions will be repelled in addition. It would seem that the intact skin, having the characteristics of a lipoid membrane, allows the passage of lipid-soluble drugs in their undissociated form while restricting the entry of the dissociated form.

The medicament must first pass through the fatty protective layer in the healthy epidermis before either reaching the keratin or penetrating the appendages. Defatting the skin does much to increase absorption assuming the medicament is absorbable, and this would account for the better absorption usually encountered from ointments incorporating surface-active materials or vehicles which defat. The vehicle must not, of course, prevent release of the medicament by any physico-chemical means.

The non-penetrability of saturated mineral oils may be explained as resulting from their general inertness. The introduction of polar groups has been shown by Harry to confer penetrant properties. This observation suggests that the passage through the membrane is not wholly mechanical; some form of active transport may also be involved. At least, physico-chemical activity is essential for the reactions which are associated with cells and interfaces.

The early workers did not have sufficient evidence to suggest that any but the simplest principles were involved. It was soon observed that defatting solvents like chloroform or ethanol enhanced penetration. But many results are anomalous because in the attempts to study release of the medicament little if any attention seems to have been given to whether the medicament could diffuse from anywhere but the surface of the base. Wolfhügel and von Knorre[96] in 1881 found that the amount of phenol liberated from a 5 per cent solution in oil was negligible, and this was clearly shown by Koch[47], who demonstrated that this preparation did not kill spores of *Bacillus anthracis* after 110 days immersion. The principle of diffusion and partition coefficient was recognised, but that diffusion of the medicament from within the base is necessary seems often to have been overlooked. Seelman[15] demonstrated this very simply by placing moistened blue litmus paper on a slide covered with official boric acid ointment. The litmus was still blue after several hours, showing that no penetration or release of acid had occurred. The work on sulphonamide release suggests that the activity of the preparations is often that of the surface layer. As Fuller and colleagues[20] showed, even in a base of glycerol-gelatin a flow rate of 20 ml./hour removed sulphonilamide faster than it could diffuse from within the base, and it was 25 hours before anything approaching a stable system resulted.

This relation between vehicle and base should be recognised, and a knowledge of the physico-chemical properties of both base and medicament should form the basis for a more fundamental approach to the problem of drug release.

The ideal would be to so tailor the medicament molecule and so choose a vehicle that all but one of the basic requirements of percutaneous absorption can be met by physico-chemical factors. Unfortunately the requirement—that of absence of other than the desired effect on the body—is the stumbling block of many a good intention, and, although a compromise would almost invariably result, preparations based on the study of recognised principles should give more reliable effects than those based on empiricism.

It would seem that a standard *in vitro* method of estimating the availability of the medicament is still required. This should be a diffusion technique of the simplest system—a standard weight and volume of medicated base available over a standard area above which is a simple fluid from which samples for assay can be withdrawn at known distances from the base.

In vivo methods must of necessity vary with the purpose of the medicament. Where activity in the skin only is required, histological methods offer the best results. Systemic effects should be assessed quantitatively by biochemical estimation or when more specific knowledge is required by a radio-active tracer technique.

References

1. Eller and Wolff, *J. Amer. med. Ass.*, 1940, **114**, 2002.
2. Rothman, *Handbuch der normalen und pathologischen Physiologie*, 1929, **4**, 107.
3. Neuroth and Lee, *J. Amer. pharm. Ass. (Pract.)*, 1945, **6**, 285.
4. Calvery, Draize and Laug, *Physiol. Rev.*, 1946, **26**, 495.
5. Busse, *J. Amer. pharm. Ass. (Pract.)*, 1943, **4**, 314.
6. Guillot, *J. Physiol., Paris*, 1954, **46**, 31.
7. Lane and Blank, *Arch. Derm. Syph.*, 1946, **54**, 497, 650.
8. Mehlhose, *Pharmazie*, 1947, **2**, 202.
9. Vallette and Cavier, *J. Physiol. Path. gen.*, 1947, **39**, 137.
10. Sollman, *Manual of Pharmacology*, 7th Ed., p. 30.
11. Hadgraft and Somers, *J. Pharm. Pharmacol.*, 1956, **8**, 625.
12. Ashley, *Austral. J. Pharm.*, 1955, **36**, 989.
13. de Roeck, *Pharm. Tijdschr. Belg.*, 1955, **32**, 233.
14. Lockie and Sprowls, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 222.
15. Seelman, *J. Amer. med. Ass.*, 1938, **110**, 1127.
16. Hawking, *Quart. J. Pharm. Pharmacol.*, 1941, **14**, 226.
17. Waud and Ramsay, *Canada Med. Ass. J.*, 1943, **48**, 121.
18. Bandelin and Kemp, *J. Amer. pharm. Ass., Sci. Ed.*, 1946, **35**, 65.
19. Howard, *New Engl. J. Med.*, 1945, **232**, 698.
20. Fuller, Hawking and Partridge, *Quart. J. Pharm. Pharmacol.*, 1942, **15**, 127.
21. Breslauer, *Z. Hyg.*, 1895, **20**, 165.
22. Cheyne, *Lancet*, 1915, **1**, 419.
23. Reddish, *Proc. Amer. Drug. Mfg. Ass.*, 1929, **16**, 116.
24. Ruehle and Brewer, *U.S. Dept. Agric., Circ.* No. 198, 1931.
25. Reddish and Wales, *J. Amer. pharm. Ass.*, 1929, **18**, 576.
26. Huyck, Hirose and Reyes, *ibid.*, 1946, **35**, 129.
27. Husa and Radin, *ibid.*, 1932, **21**, 861.
28. Pillsbury, Livingood and Nichol, *Arch. Derm. Syph.*, 1942, **45**, 61.
29. Rae, *Brit. J. Derm. Syph.*, 1944, **56**, 92.
30. Luff., *Pharm. J.*, 1891, **50**, 206.
31. Coran and Huyck, *J. Soc. cosmet. Chem.*, 1956, **7**, 20.
32. Izgu and Lee, *J. Amer. pharm. Ass. (Pract.)*, 1954, **15**, 396.

33. Aymer and Ferlauto, *J. Amer. pharm. Ass., Sci. Ed.*, 1947, **36**, 211.
34. Zheutlin and Fox, *J. Invest. Derm.*, 1948, **11**, 161.
35. Clark and Davies, *J. Pharm. Pharmacol.*, 1949, **1**, 521.
36. Velu, Claude, Peyre and Viennet, *Ann. pharm. franç.*, 1953, **11**, 675.
37. Magner and O'Sullivan, *Canad. med. Ass. J.*, 1944, **50**, 118.
38. Legroux, *Mem. Acad. Chim.*, 1940, **13**, 415.
39. Zondek, Bromberg and Shapiro, *Proc. Soc. exp. Biol., N.Y.*, 1942, **50**, 116.
40. Woodward, Wright, Evenson, Ofner, Kramer, Jenner and Johnson, *Fed. Proc.*, 1944, **3**, 87.
41. Meyers, Nardkarni and Zopf, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 231.
42. Lund, *Arch. Pharm. Chem.*, 1951, **58**, 595.
43. Cole et al., *Arch. Derm. Syph.*, 1928, **17**, 625.
44. Laug, Vos and Kunze, *J. Amer. pharm. Ass., Sci. Ed.*, 1947, **36**, 14.
45. Laug and Kunze, *J. Ind. Hyg. Toxicol.*, 1948, **30**, 256.
46. Brodie and Hogben, *J. Pharm. Pharmacol.*, 1957, **9**, 345.
47. Hunter and Smith, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 125.
48. Macht, *J. Amer. med. Ass.*, 1938, **110**, 409.
49. Walzer, *Arch. Derm. Syph.*, 1940, 41, 692.
50. Zondek, *Klin. Wschr.*, 1929, **8**, 2229.
51. Moore, Lamar and Beck, *J. Amer. med. Ass.*, 1938, **111**, 11.
52. Nelson, Greene and Wells, *Endocrinology*, 1940, **26**, 651.
53. Hadgraft, Somers and Williams, *J. Pharm. Pharmacol.*, 1956, **8**, 1027.
54. Hadgraft and Somers, *ibid.*, 1954, **6**, 944.
55. Zondek, *Lancet*, 1938, **1**, 1107.
56. Pichotka and Mayer, *Arzneimitt.-Forsch.*, 1954, **4**, 277.
57. Lucas and Guth, *J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 344.
58. Brockemeyer and Guth, *ibid.*, 1955, **44**, 706.
59. Zondek, *Nature, Lond.*, 1942, **149**, 334.
60. Green, *Quart. J. Pharm. Pharmacol.*, 1946, **19**, 107.
61. Strakosch, *Arch. Derm. Syph.*, 1944, **49**, 1.
62. Duemling, *ibid.*, 1941, **43**, 264.
63. Eller and Wolff, *ibid.*, 1939, **40**, 900.
64. Clark, Strakosch and Nordlum, *Proc. Soc. exp. Biol., N.Y.*, 1942, **50**, 43.
65. Strakosch and Clark, *Amer. J. med. Sci.*, 1943, **20P**, 518.
66. Strakosch and Clark, *ibid.*, 1943, **206**, 610.
67. Harry, *Brit. J. Derm. Syph.*, 1941, **53**, 65.
68. Butcher, *J. Invest. Dermatol.*, 1953, **21**, 243.
69. Seki, *Japan. J. Pharm. Chem.*, 1951, **23**, 138.
70. MacKee, Hermann, Baer and Sulzberger, *J. Lab. clin. Med.*, 1943, **28**, 1642.
71. Cullumbine, *Brit. J. Derm Syph.*, 1946, **58**, 291.
72. Helander, *Nature, Lond.*, 1945, **155**, 109.
73. Wild, *Brit. med. J.*, 1911, **1**, 161.
74. Wild and Roberts, *ibid.*, 1926, **1**, 1076.
75. Moncorps, *Arch. exp. Path. Pharmak.*, 1929, **141**, 25.
76. Moncorps, *ibid.*, 1931, **163**, 26.
77. Brown and Scott, *J. Pharmacol.*, 1934, **50**, 32, 373.
78. Nadkarni, Meyers, Carney and Zopf, *Arch. Derm. Syph.*, 1951, **64**, 294.
79. Shelley and Melton, *Fed. Proc.*, 1947, **6**, 199.
80. Jacobi and Lantzsch, *Pharm. Zentralh.*, 1952, **91**, 6.
81. Barker, Christian and De Kay, *J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 601.
82. Johnson and Lee, *ibid.*, 1943, **32**, 278.
83. Czetsch-Lindenwald, *Pharm. Ind.*, 1943, **10**, 29.
84. Szczesniak, Sherman and Harris, *Science*, 1951, **113**, 293.
85. Cyr, Skauen, Christian and Lee, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 615.
86. Skauen, Cyr, Christian and Lee, *ibid.*, 1949, **38**, 618.
87. Lux and Christian, *Amer. J. Physiol.*, 1950, **162**, 193.
88. Urakami and Christian, *J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 179.
89. Loeffler and Thomas, U.S. Naval Radiol. Defense Lab., AD-225(*b*), 1950, p. 25.
90. Severan and Tack, *Pharm. Tijdschr. Belg.*, 1952, **29**, 41.
91. Edwards, *Nature, Lond.*, 1954, **173**, 1042.
92. Lange and Evans, *Radiology*, 1947, **48**, 514.
93. Graul, *Strahlentherapie*, 1953, **92**, 197.
94. Witten, Ross, Oshry and Hyman, *J. Invest. Dermatol.*, 1951, **17**, 311.
95. Witten, Bauer, Holmstrom and Loevinger, *ibid.*, 1953, **20**, 93.
96. Wolfhugel and von Knorre, *Mitt. Kaiser Gesundheit*, 1881, **1**, 635.