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LITERATURE SURVEY

Topical Drug Delivery to Cattle and Sheep

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Keyphrases □ Topical drug delivery—to cattle and sheep, literature survey □ Cattle—topical drug delivery, literature survey □ Sheep—topical drug delivery, literature survey

CONTENTS

Twenty-one years ago, Rogoff and Kohler (1) demonstrated that the application of 100 ml of a concentrated solution of the organophosphorus insecticide crufomate (I) (Table I) to a small area of a cow's skin could control cattle grubs. Compound I is a systemic insecticide (2), and its mode of action is believed to involve its absorption across the skin into the cow's systemic circulation and, hence, distribution throughout the body, thus interacting with cattle grubs as they migrate through the tissues. Confirmation of drug absorption came from the observation that a slight depression of erythrocyte cholinesterase activity followed drug administration (1). This type of dosage form is referred to as a "pour-on" or "spot-on". It is a liquid formulation (solution, emulsion, or suspension) intended to be applied to a small area of an animal's skin to promote drug absorption into the bloodstream. The concentration of drug in a spot-on is higher than that in a pour-on; consequently, a smaller dosage is used.

The realization that drugs can be introduced topically into the bloodstream has stimulated the search for topical drug delivery systems to control internal and external parasites and to deliver nutrients, metabolic regulators, and hormonal regulators.

Systemic insecticides can be used to control external parasites if the latter are bloodsuckers (*e.g.*, sucking louse *Linognathus vituli*) or if drug is secreted onto the skin *via* sweat or sebaceous glands.

Although the goal of delivering drugs to the systemic circulation *via* the topical route has only been actively researched during the past 21 years, the farm industry has many years of experience in the application of drugs to the hair, wool, and skin of domestic animals by way of dips, dusts, and sprays. However, these dosage forms usually contain a much smaller drug concentration than dosage forms such as pour-ons that are intended to promote systemic delivery. Consequently, because the driving force for Fickian diffusion across a membrane such as skin is the concentration gradient across the membrane, these dosage forms usually act by bringing the drug into contact with an external parasite rather than by promoting absorption.

For example, it is common practice to treat cattle grubs in beef cattle with a dip containing 0.25% I or a spray containing 0.375% I. The usual strengths of the pour-ons and spot-ons of I are 8 and 12%, respectively. Similarly, it is common practice to treat horn flies (*Haematobia irritans*) in beef cattle with back rubbers[1] (burlap soaked with a mixture of an insecticide in an oil such as diesel fuel wrapped around a wire or cable) containing 1% coumafos (II) (3), dust bags[1] (doubled burlap bags containing powdered drug) containing 1 or 5% II, sprays containing 0.06%

[1] They apply drug to the animal's coat when the animal rubs against them.

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Table I—Some Veterinary Antiparasite Drugs [a]

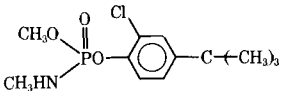
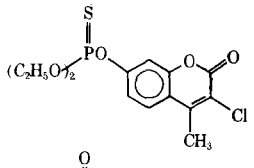
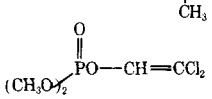
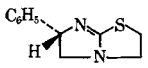
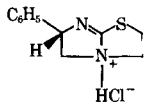
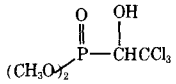
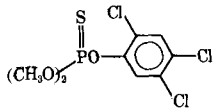
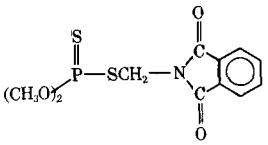
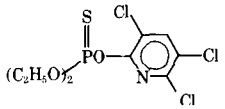
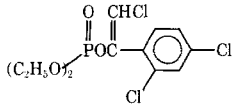
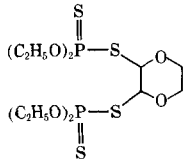
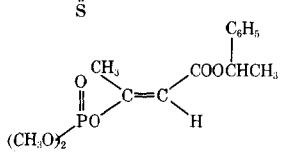
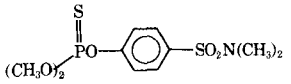
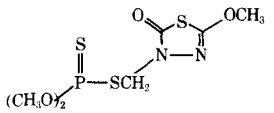
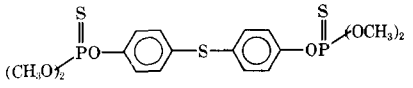
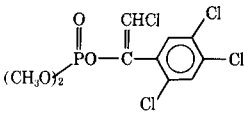
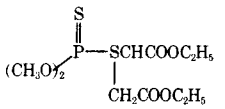
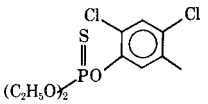
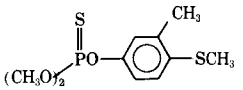
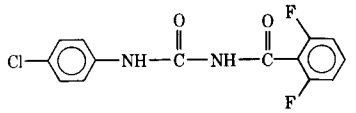
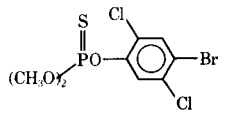
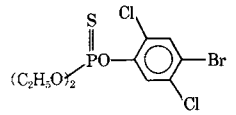
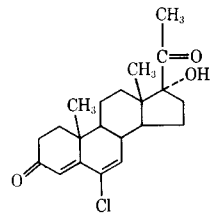
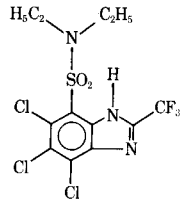
Compound Number	Generic Name	Trade Names	Structure	Melting Point	Solubility: Water	Solubility: Organic Solvents
I	Crufomate	Dowco 132, Montrel, Ruelene		60–60.5°	pi[b]	S[c]: acetone, benzene, carbon tetrachloride; pi: light petroleum
II	Coumafos	Co-ral, *etc.*		91°	pi	PS[d]: acetone, chloroform, corn oil
III	Dichlorvos	Atgard, *etc.*		Liquid	1 g/100 ml	M[e]: alcohol, most nonpolar molecules
IV	Levamisole	Levasole, Nemicide, Citarin-L		60–61.5°	—	—
V	Tetramisole hydrochloride	Citarin, Nilverm ± isomer of IV		264–265°	21 g/100 ml	S: methanol, propylene, glycol; SpS[f]: ethanol; SS[g]: chloroform, hexane, acetone
VI	Trichlorfon	Dylox		83–84°	15.4 g/100 ml	Chloroform: 75 g/100 ml; ether: 17 g/100 ml; benzene: 15.2 g/100 ml; VSS[h]: pentane, hexane
VII	Ronnel	Viozene		41°	0.004 g/100 ml	FS[i]: acetone, carbon tetrachloride, ether, methylene chloride, kerosene
VIII	Phosmet	Imidan		71.9°	25 ppm	Organic solvents
IX	Chlorpyrifos	Dowco 179		1–42°	2 ppm	Isooctane: 79% W/W; methanol: 43% W/W; FS: other organics
X	Chlorfenvinphos	—		Liquid	VSpS[j] (dec. at pH >7)	FS: organics
XI	Dioxation	Navadel		Liquid	pi	PS: hexane
XII	Crotoxyfos	Ciodrin		Liquid	0.1%	SS: kerosene, saturated hydrocarbons; S: acetone, chloroform, ethanol, highly chlorinated hydrocarbons
XIII	Famophos	Warbex		52.5–53.5°	—	—
XIV	Methidathion	Supracide		39–40°	<1%	S: most organics
XV	Temefos	Lapor			—	

Table I—*Continued*

Compound Number	Generic Name	Trade Names	Structure	Melting Point	Solubility	
					Water	Organic Solvents
XVI	Tetrachlorvinfos	Rabon		97–98°	11 ppm	Xylene: <15%; chloroform: 40–50%
XVII	Malathion	Malamar 50, Prioderm		2.9°	145 ppm (hydrolyzed pH >7 or <5)	Soluble in most organics
XVIII	Dichlofenthion	Nemacide, Bromex		Liquid	SS	M: most organics
XIX	Fenthion	Tiguvon		Liquid	55 mg/liter	M: most organics
XX	Difluron	—		—	—	—
XXI	Bromopfos	—		—	—	—
XXII	Ethyl bromopfos	—		—	—	—
XXIII	Chlormadinone	—		—	—	—
XXIV	4,5,6-Trichloro-7-(diethylsulfamoyl)-2-(trifluoromethyl) benzimidazole	—		—	—	—

[a] From "The Merck Index" 9th ed., Merck & Co., Rahway, N.J., 1976. [b] pi = practically insoluble. [c] S = soluble. [d] PS = partially soluble. [e] M = miscible. [f] SpS = sparingly soluble. [g] SS = slightly soluble. [h] VSS = very slightly soluble. [i] FS = freely soluble. [j] VSpS = very sparingly soluble.

II (3), or dips containing 0.06% II. The usual strength for a pour-on of II is 4%.

Another example of a drug delivery system that acts primarily by bringing drug into contact with the pest rather than promoting absorption is polyvinyl chloride strips impregnated with dichlorvos (III) and attached to the legs of cattle to control cattle grub by killing ovipositing flies, eggs, or newly hatched larvae (4).

Although these dosage forms are not necessarily intended to promote systemic absorption, they may do so with harmful effects to the animals under abnormal conditions. For example, if sheep are driven too far or too fast or are confined to a woolshed to protect them from rain after dipping, overheating can occur, thereby increasing the skin temperature sufficiently to promote the absorption of the dip chemicals (5).

Most of this review will be concerned with topical delivery of drugs into the systemic circulation rather than with delivery onto the skin to promote local contact with ectoparasites. However, an understanding of the permeability of cattle and sheep skins to drugs will provide a basis for preventing drug absorption (thus reducing toxicity to the host animal), prolonging drug action, and promoting drug delivery.

The topical route of systemic delivery is attractive to the farming industry because:

1. It is less labor intensive to apply a drug to an animal's skin than to administer more conventional dosage forms such as drenches, injections, and inocculations.
2. The drug is introduced into the bloodstream without

having to traverse the GI tract where it can be extensively metabolized or bound to proteins and other GI tract contents or where its transit time is unpredictable.

3. The dose for each animal can be regulated much more closely than when drugs are added to feed or are presented as licks.

4. When properly formulated, these systems are likely to cause less trauma and tissue damage than injections or drenches and are less likely to interfere with nutritional status.

The pour-on or spot-on dosage form is particularly attractive since it can be applied rapidly and easily to an animal's skin and the cost of equipment is much less than that required for spraying or dipping. However, it has been argued that the pour-on technique can be more expensive than spraying when large numbers of cattle are to be treated, the application lacks the shampooing effect of a shower treatment, and the operator is exposed to the grave risk of absorbing toxic materials from handling concentrated drug products that have been specifically formulated to promote absorption (6). This latter point was recently investigated (7), with the conclusion that experienced veterinarians can use commercially available organophosphorus pour-ons without absorbing enough drug to depress blood cholinesterase activity. This safety aspect may not be so high with inexperienced operators.

It has long been recognized that topical drug administration to humans has many advantages. As the result of a workable understanding of the barrier properties and transport mechanisms of human skin and of the influence of the physicochemical properties of drugs, vehicles, and other formulation excipients on the absorption process, pharmaceutical scientists can now produce topical dosage forms for humans that demonstrate well-controlled drug delivery. Some excellent reviews were written on these subjects (8–12). The discovery that the cells of the human stratum corneum, the dead keratinized epithelium that comprises the outer 0.001 cm of the skin, are the rate-determining barrier to human skin absorption has led to the design and performance of numerous *in vitro* experiments using isolated skin.

At present, the barrier properties of sheep and cattle skins are not well understood. One report (13) suggested that drugs are much more likely to traverse sheep and cattle skins by way of skin appendages (*i.e.*, hair follicles, sweat and sebaceous glands, and ducts) rather than through the cells of the stratum corneum as in humans, in spite of the fact that both sheep (14) and cattle (15) skins have a substantial stratum corneum. However, more work is necessary to clarify this point.

The next section of this review describes the gross structure of cattle and sheep skins and the physicochemical properties that these barriers are likely to present to diffusing drug molecules. This section is followed by a discussion of the possible changes in the barrier properties of skin as the result of season, temperature, age, sex, humidity, *etc.* Another section is concerned with useful parameters for understanding the permeability character-

Table II—Thickness of Cattle and Sheep Skins

Animal	Breed	Body Region (Season)	Thickness, mm					Reference
			Whole Skin	Stratum Corneum	Uncornified Epidermis	Papillary Layer of Dermis	Reticular Layer of Dermis	
Cattle	Ayrshire Bullock	D T-L[a] (winter)	—	3.09×10^{-2}	3.11×10^{-2}	—	—	15
	Devon	Midside (unknown)	8.15	—	—	—	—	36
	Hereford	Midside (unknown)	6.77	—	—	—	—	36
	Zebu Cross	Midside (unknown)	6.43	—	—	1.40	4.56	36
	Australian Illawarra Shorthorn	Midside (unknown)	6.23	—	—	—	—	36
	Friesian	Midside (unknown)	6.08	—	—	—	—	36
	Zebu	Midside (unknown)	5.77	—	—	0.98	4.45	36
	Aberdeen Angus	Midside (unknown)	5.75	—	—	—	—	36
	Shorthorn	Midside (unknown)	5.69	—	—	1.70	4.08	36
	Jersey	Midside (unknown)	5.46	—	—	—	—	36
	Jersey	Upper thorax (summer)	3.5	—	—	—	—	37
	Jersey	Upper thorax (spring)	4.7	—	—	—	—	37
	Sahiwals	Upper thorax (summer)	4.7	—	—	—	—	37
	Sahiwals	Upper thorax (spring)	5.7	—	—	—	—	37
Sheep	Finnish Landrace/Dorset Horn	D T-L (winter)	—	3.14×10^{-2}	1.67×10^{-2}			
	Southdown	Various (unknown)	2.7	—	—	—	—	22
	Shropshire	Various (unknown)	—	—	2.7×10^{-2}[b]	—	—	22
	Merino	Various (unknown)	—	—	4.2×10^{-2}[b]	—	—	22

[a] Dorsal thoraco-lumbar. [b] Reported as average total epidermal thickness.

istics of membranes and the conclusions about cattle and sheep skin permeability that can be drawn from some *in vitro* studies. The review concludes with an examination of reports of field and laboratory experiences with veterinary topical drug delivery systems (primarily pour-ons and spot-ons) and suggestions for future work.

THE BARRIERS

Hair and Wool—The first absorption barrier encountered by drug molecules following their application to the skins of cattle and sheep is the hair or wool coat. There are ~2000 hair fibers/cm^2 in cattle (16, 17), although this number is affected by the animal's breed, sex, weight, age, and body region. Each fiber grows out of hair follicles, which, in cattle, are randomly distributed over the animal's body. Each follicle has a sweat gland, a sebaceous gland, and an arrector pili muscle associated with it. The hairs of European cattle leave the skin at an average angle of 61–62° (18), whereas those of Asian and African cattle tend to leave at an average angle of slightly less than 60° (19).

The hair and wool follicles in sheep, and consequently the hair and wool fibers, are not randomly distributed over the body but are in definite groups containing one to five primary follicles (20), which have a sebaceous gland, a sweat gland, an arrector pili muscle, and a number of secondary follicles, which have only sebaceous glands. Merino sheep were reported to have 300–400 primary follicles/cm^2 and 6000–10,000 secondary follicles/cm^2 in wool-growing regions (*i.e.*, midside, midback, and flank) of the body (21). The average density of primary follicles does not change appreciably on the hair-growing regions, but the densities of secondary follicles are markedly reduced. Differences in follicle densities and in ratios of numbers of primary and secondary follicles with breed were discussed elsewhere (20, 21).

Because hair and wool fibers are composed of the modified protein keratin, they possess chemically reactive groups such as thiol, amino, and carboxyl groups and hydrophobic regions. Thus, they can react with and change (frequently reduce) the thermodynamic activity of drugs that they contact. Many chemicals bind strongly to wool (23–28), and a similar situation must be expected with cattle hair.

However, sheep wool and, to a lesser extent, cattle hair are coated with an emulsion of sweat and sebum that is formed in the follicle infundibula where the sweat and sebaceous glands empty their secretions. Wool fibers appear to have an almost continuous coat of this emulsion (29) [frequently referred to as the yolk (30)], while only the lower parts of cattle hairs are similarly coated (29). This emulsion rapidly dissolves many chemicals that are applied to the coat or skin of animals (31, 32), and diffusion within the emulsion, either up or down the fibers, always competes with diffusion of molecules through the skin (33, 34).

Consequently, an understanding of the nature of the emulsion and the manner in which it varies with breed, sex, climate, season, and nutritional state is fundamental to an understanding of topical drug delivery.

Skin Topography—Scanning electron micrographs of cattle and sheep skins reveal that the surfaces consist of roughly hexagonal squama punctuated by hair or wool fibers and their associated follicle pores (29). The pores are not open and clear but are protected by a conical mass, which appears to be composed of squama and a convex amorphous material. The latter is believed to be a partially dehydrated or modified form of the emulsion of sweat and sebum that is formed in the follicle infundibula. As mentioned previously, the convex amorphous material also coats most of the wool fibers and the lower parts of cattle hairs. Globules or strips of this material are also present at the junctions of the interfollicular squama in both cattle and sheep skin; in the latter, a significant amount is present on the squama surfaces.

Gross Structure of Cattle and Sheep Skins—Like other mammals, the skin of cattle (35) and sheep (20, 22) consists of a cornified stratum corneum, an uncornified "living" epidermis, and a dermis differentiated in the cow and, to a much smaller extent, sheep (20) into papillary and reticular layers. The relative thicknesses of the various skin layers in different animal breeds are given in Table II.

The most reliable studies of the structure of the stratum corneum have employed cryostat sections (14, 15). This technique causes much less damage to the stratum corneum than does a histological technique, which involves dehydration and embedding in paraffin.

The stratum corneum of cattle appears to consist primarily of vertical columns of cells (15). The outer two-thirds of the stratum corneum cells, but not the lower third, the uncornified epidermis, or the sweat glands, are permeated by an emulsion believed to be the emulsified sweat mentioned earlier. It is proposed that the emulsion is formed in the follicle infundibula and oozes to the surface through the outer layers of the stratum corneum and the follicle pore (15).

The entire stratum corneum of sheep is permeated by emulsion, and an intact film of emulsion ~0.90×10^{-2} mm thick covers its outer surface (14). The lower half of the stratum corneum (1.6×10^{-2} mm) consists of vertical columns of densely packed flattened cells, whereas the outer half consists of a loosely knit structure.

The papillary layer of the dermis has a depth of 1–2 mm in cattle (Table II) but is much thinner in sheep (20). It is composed of fine collagenous fibers interwoven with fine elastic and reticular fibers. The reticular layer is a broad zone of coarse collagenous fibers. Goldsberry and Colhoun (38) suggested that the transition zone between the papillary and reticular layers of dermis in cattle occurs around the hair follicle bulb, whereas Jenkinson (35) suggested that the two layers meet in the region of the sebaceous gland. In any case, in both sheep and cattle, the sebaceous gland is associated with the upper portions of the hair follicle and the sweat glands are below the follicle bulb (22, 35). In both species, the ducts of the sweat glands open into the infundibula of the hair follicles (primary follicles in sheep) slightly above the entry point of the sweat ducts (22, 35, 38).

The blood supply to the skin is particularly important to drug delivery because once the drug comes into contact with a capillary network, it has the opportunity of entering the blood and being carried away from the skin. Entry into the blood is likely to be rapid unless the drug molecule is very hydrophobic (39).

Blood supply to mammalian skin was reviewed previously (35). Briefly, the hair bulb, the sebaceous gland, the

sweat gland, and especially the hair follicles are richly supplied by blood in both cattle and sheep. These blood vessels also permeate the papillary layer of the dermis.

Emulsion—It was previously stated that the hair and wool fibers, the infundibula of the hair and wool follicles, the junctions of squama of the stratum corneum, and all (in the case of sheep) or part (cattle) of the stratum corneum are permeated by an emulsion which oozes up from the follicle infundibula. The chemical components of the emulsion vary from animal to animal and with such factors as the time of year and climate, but most lipids in the emulsion arise from the secretions of the sebaceous glands (14, 15, 40). Minor components are likely to arise from desquamating squama (*i.e.*, keratin), cell debris, bacterial excreta, and blood. The likely components of the emulsion are listed in Table III (41–43). Thus, the solvent properties of the emulsion and its ability to solubilize and modify the thermodynamic activity of topically applied drugs will be determined by the relative concentrations of the various constituents.

In addition, the viscosity of the emulsion and thus its ability to support molecular diffusion will vary with its water content. This latter parameter will be influenced by the activity of the sweat gland, but it also has been inferred (29) that the emulsion may lose water by evaporation as it permeates through the layers of the stratum corneum. Future physicochemical studies must elucidate the solubility characteristics of skin emulsions under conditions likely to be encountered by sheep and cattle.

BARRIER VARIABILITY

Exposure to Solvents—Lloyd *et al.* (44) showed that cattle skin can be clipped coarsely or finely without removing more than four cell layers of the stratum corneum. However, washing the skin with ether removed all but three stratum corneum layers (44). This effect is almost certainly involved in the marked erythema that ether causes on bovine skin (45), and it predisposes the skin to infection by *Dermatophilus congolensis* (46). Methanol washing of cattle skin had a much smaller effect on stratum corneum thickness (44) and on the incidence of infection (46). The susceptibility of cattle skin to infection also was increased by washing with light petroleum (47) or following intense water spraying (48); in sheep skin, it was increased by heavy rain (49).

The development of erythema and susceptibility to infection probably are caused by removal of the outer squama of the stratum corneum and, in particular, removal of the emulsion from the skin surface, from the junctions of squama, and from hair pores. Consequently, treatment of skin with these solvents, as well as with detergents, is likely to affect its permeability, and the extent to which this occurs is worthy of investigation.

Lipid solvents and dimethyl sulfoxide treatment of cattle and sheep skin at 20° apparently stimulates the secretory cells of the sweat glands and promotes cutaneous water loss (50). Hence, as well as removing skin lipids, the application of organic solvents to the skin is likely to lead to increased hydration of the stratum corneum and changes in the composition and viscosity of any emulsion that remains associated with the skin.

The resistance of cattle skin to infection returned 24 hr after washing with ether (46), although only one lipid layer (*i.e.*, layer of stratum corneum cells) had been replaced in that time (44). It is thus likely that replacement of emulsion in the junctions between the squama is well underway by 24 hr (46).

Table III—Major Constituents of Skin Emulsion

Source	Constituents
Sebaceous gland	Monoester waxes, diester waxes, squalene, cholesterol, cholesterol esters, phospholipids, triglyceride esters of fatty acids, free fatty acids, corticosteroids
Sweat gland	Water, sodium ions, potassium ions, calcium ions, magnesium ions, chloride ions, lactate ions, free amino acids, water-soluble proteins, epinephrine
Blood	Albumin, transferrin, immunoglobulins IgA, G, and M

The precise effects of these changes cannot be predicted at this stage. They will depend on the mechanism by which drug molecules are absorbed and on the chemical properties of each drug.

Skin Temperature—Elevation of temperature increases the molecular diffusion rate by lowering its activation energy. However, temperature changes also influence skin permeability by changing the activity of sweat glands and the composition of their secretions. The results of these changes are expressed as changes in the composition and viscosity of the skin emulsion and in the degree of skin hydration.

The temperature of sheep and cattle skins is influenced by ambient temperature, radiation, convection, evaporation, shivering, and exercise (43). The skin surface temperature of cattle in the shade does not vary appreciably from one area of the body to another (43). However, the coat type and skin color are important when the animal is in sunlight. Thus, an 8° temperature difference has been reported between black-haired (heat-absorbing) and white-haired (heat-reflecting) regions (51).

The effect of ambient temperature on domestic animal skin permeability is likely to be more profound than it is on human skin. This difference arises partly because humans, especially those receiving dermal treatment, are unlikely to expose their skin to the wide range of temperatures (*e.g.*, 0–50°) that animal skins may be exposed to. In addition, animal skin temperatures tend to change much more dramatically with changing environment than do human skin temperatures. For example, the surface temperature of cattle was observed (52) to vary from 34.5 to 40° when ambient temperature was increased from 15 to 40°. The temperature of the skin of sheep varied from 31 to 38.5° when ambient temperature was varied from 20 to 40°. Rectal temperatures varied from 38.2 to 40.2° throughout the same range of ambient temperatures.

The greater efficiency of the human skin thermoregulatory process could arise because of the greater rate of human sweating (2000 g^{-2}/hr) as against sheep (32 g^{-2}/hr) or cattle (588 g^{-2}/hr) (53).

Increases in ambient temperature increase the rate and volume of secretion of sweat in cattle (54, 55) and in shorn (53, 56) and unshorn (56) sheep. This factor may profoundly affect the aqueous content and viscosity of the emulsion because heat has only a minor effect on the output or composition of sebum (57) unless it is prolonged for >3 days (57, 58).

In addition to the higher output of sebum when high

temperatures were maintained for long periods, the relative concentration of linoleic acid (and other free fatty acids) in the sebum increased (57, 58). The importance of this finding to dermal drug delivery results from the fact that linoleic acid reduced the increased water loss from the skin of rats that was induced by fatty acid deficiency (59, 60) and is thus likely to affect skin permeability to other compounds.

Low humidity at a constant temperature caused an increase in the concentrations of palmitic and myristic acids in sebum of cattle (57).

Season—Most changes observed in the structure and anatomy of animal skins as a result of seasonal changes follow a predictable pattern. Smith and Jenkinson (45) found that the sebum output by Ayrshire cattle in Ayre was slightly lower in winter than at other times of the year. However, the situation may be different in hot climates because it was reported (61) that sebum secretion in cattle (Shorthorns, Friesans, and Egyptian cattle) in Egypt was greater in winter than in summer. Similarly, Nay and Hayman (62) reported that the sweat gland volume, as well as the papillary layer and skin thickness of cattle, was greater in winter than in summer. The increased size of the sweat gland indicates that it is less active in winter than in the summer (63). Amakiri (64) reported that the thickness of the total skin, the stratum corneum, and the epidermis of cattle in Nigeria was slightly thicker during the rainy season than during the dry season. The direct relationship between sweat gland volume and papillary layer thickness observed for cattle appears to be quite general (62).

Breed—Table II reveals differences in both total skin thickness and thickness of various skin layers for cattle of different breeds.

Differences also occur in the density of hair follicles with different breeds of cattle and sheep. The hair follicle density of a large number of breeds of cattle with different body weight, age, sex, and growth rate follows the rule (65):

$$D = kW^{-0.67} \qquad \text{(Eq. 1)}$$

where D is the density of hair follicles and W is the animal weight. The constant k varies slightly with breed. It was observed that the density of hair follicles for Hereford and Shorthorn cattle was similar to those of Africander crossbreeds provided all the animals had similar weights (65). However, Brahman cattle had 20% more follicles per unit area than animals of the other breeds with similar weights.

The rule expressed in Eq. 1 accounts for the observations that cattle between 1 and 2 years of age had denser coats than older cattle of the same breed and that coats were densest in times of drought (*i.e.*, when the animals were lighter) (66).

Differences in follicle densities and in ratios of the number of primary to secondary follicles in different breeds of sheep have been studied (20, 21). Jenkinson and Nay (18, 19) found that the ratio of length (L) to diameter (D) of sweat glands (L/D) and the depths of the hair follicle (FD) varied in a regular manner between breeds of cattle. These authors characterized three skin types: Type 1, where $L/D < 8.0$ and $FD < 1.5$ mm (Jersey cattle and most Asian and African cattle); Type II, where $L/D > 12$ and $FD > 2$ mm (many Scottish highland breeds); and Type III, where $L/D > 12$ and $FD < 1.5$ mm (some South American breeds).

Tropical cattle (*e.g.*, Asian, African, and South American breeds) frequently have smaller sweat gland volumes than European breeds (19, 64), perhaps because the glands are more active. Values of various average parameters for cattle from different continents are given in Table IV.

The complexity of this topic is illustrated by the observation that Hereford × Shorthorn cattle sweated at a higher rate than Brahman × Shorthorns under mild conditions but at a lower rate when under stress (67).

Body Region—The hair and wool follicle densities and types of fibers in sheep and cattle vary significantly with the region of the body examined. The body of a sheep can generally be divided into wool-growing regions and hair-growing regions. The approximate location of these regions in numerous breeds is described in Table V (20). The epidermis of the skin is thinner on wool-growing areas (30 μm) than in hair-growing regions (500 μm) (20). Similarly, while there is an area of uniform skin thickness in the middle of a sheep's back on either side of and parallel to the vertebral column (22), there is also a steep dorsoventral gradient in skin thickness on either side of the medial dorsal line. The thicker skin is over the vertebral column, near the tail along the back of the sheep and towards the neck in older sheep.

As already mentioned, the wool follicles can be primary (with a sweat gland and a sebaceous gland) or secondary (with a sebaceous gland only). It was reported (20) that the sebaceous and sweat glands are larger in the hair- than in the wool-growing regions.

These observations suggest that whether drugs cross sheep skin *via* the cells of the epidermis or *via* the skin appendages, the permeability of the skin of wool-growing areas (thinner epidermis and more follicles) would be greater than that of haired skin. One factor that could reverse this expectation would be the greater potential of the dense wool coat, relative to the thinner hair coat, to react with applied drugs.

A study of the density of sweat glands and, thus, of hair follicles of Ayrshire cattle (16) indicated that the highest density (2500 cm^{-2}) was in skin of the axilla and neck and that the lowest was in the lower limbs (1000 cm^{-2}). Table VI describes the areas that had above or below average density of sweat glands and area of secretory surface per square centimeter of skin.

The thickest epidermis on Hereford and Aberdeen Angus cattle was found to be where the follicle density was lowest (*e.g.*, the epidermis of the muzzle was 150–200 cells thick (38). The thinnest epidermis in Herefords was located laterally on the thorax, neck gluteal region, and upper forelegs. In three of four Aberdeen Angus, the thinnest epidermis was on the dorsal part of the body. Such information is useful for predicting the best area of an animal's body for appropriate drug application.

Other Factors—Various additional factors are likely to influence skin permeability, and more factors probably will be revealed by carefully controlled studies of skin absorption.

The influence of sex has not been explored, although it was reported that sebum output was greater in castrated male Ayrshire calves than in females (45) and that male

Table IV—Sweat Glands and Hair Follicles of Cattle [a]

Parameter	European	Asian	African	South American
Sweat gland length, μm	928 ± 276	738 ± 87	695 ± 223	972 ± 394
Sweat gland diameter, μm	107 ± 21	87 ± 18	85 ± 18	90 ± 15
L/D [b]	8.8 ± 2.5	8.6 ± 3.1	8.4 ± 3.0	10.8 ± 3.9
Sweat gland volume, $\mu m^3 \times 10^{-6}$	9.0 ± 5.2	4.9 ± 3.7	4.3 ± 2.5	6.7 ± 4.3
Follicle length, mm	2.0 ± 0.3	1.5 ± 0.3	1.6 ± 0.2	1.7 ± 0.2
Follicle diameter, μm	44.2 ± 8.1	47.3 ± 8.0	53.5 ± 7.3	54.2 ± 8.1
Follicle depth, mm	1.8 ± 0.3	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
FL/FDM [c]	46.4 ± 9.7	32.4 ± 9.1	30.2 ± 6.6	32.5 ± 5.9

[a] Data from Refs. 18 and 19. [b] Sweat gland length/sweat gland diameter. [c] Follicle length/follicle diameter.

Aberdeen Angus cattle had thicker skins than females (38).

The nutritional status of the animal is also likely to be important. Thus, when wheat-fed sheep had their diet supplemented abomasally with methionine, there was a thickening of the epidermis, poor formation and improper keratinization of wool fibers, and, subsequently, a gross thickening of the outer root sheath (68). Similarly, it was reported (69) that vitamin A deficiency in cattle resulted in hyperkeratosis, leading to substantial skin thickening in the upper two-thirds of the animal's body. The vitamin A deficiency in this case was believed to be caused by ingestion of chlorinated naphthalenes, which were used as lubricants in animal food-processing equipment.

ABSORPTION MECHANISMS

There is abundant evidence (Table VII) that many chemicals are transported across sheep and cattle skins in sufficient amounts and at sufficient rates to bring about expected pharmacological responses. However, none of the studies involving topical drug delivery to live animals provided solid evidence about absorption mechanisms. In addition, while some data are available on drug transport across excised sheep (70) and cattle (13, 71–74) skins in *in vitro* experiments, few reliable correlations were found between *in vitro* permeability parameters and *in vivo* behavior. Consequently, any speculations about absorption mechanisms at this time are highly tenuous. However, such speculations are useful in that they may create guidelines for the design and execution of needed investigations into this subject.

The speculations that follow are based mainly on knowledge of the mechanisms of drug transport across human skin (10–12, 76, 77), knowledge of the comparative anatomy and physiology of cattle and sheep skins, and conclusions drawn from a limited number of *in vitro* measurements of skin permeability (13, 70–74). The major speculations or hypotheses about cattle and sheep skin penetration are:

1. Bulk transport of neutral molecules with small to medium size molecular weights occurs largely *via* skin appendages (hair or wool follicles and associated ducts and glands) rather than the transcellular pathway which predominates in human skin penetration.

2. The rate and extent of drug absorption across the skin is significantly influenced by the composition and physical properties of the sebum–sweat emulsion associated with the skin.

Table V—Wool- and Hair-Growing Regions in Sheep

Regions	Body Area
Wool growing	Midside, midflank, flank
Hair growing	Inguinal junction, inguen, axilla, scrotum, cheek, chin, dorsal nose, manus (proximal and distal), pes, pinna

Most neutral molecules cross human skin by passive diffusion through cells of the interfollicular region rather than through the skin appendages (hair follicles and sweat ducts) (10, 12), even though neutral molecules with small to medium molecular weights have greater diffusivity in the appendages than in the stratum corneum. [The diffusion constants for water were reported to be $0.3–1.2 \times 10^{-5}$, $6–12 \times 10^{-5}$, and 2×10^{-10} cm^2/min in human hair follicles, sweat ducts, and stratum corneum, respectively (10).] It is believed that bulk transport across human skin occurs *via* the skin cells rather than the appendages because the surface area occupied by the latter is only 10^{-3} of the surface area of the skin (12).

It seems reasonable that transappendagial transport would be more important in transport across cattle and sheep skins because they contain a higher density of appendages than human skin. While a square centimeter of human skin contains an average of 40–70 hair follicles and 200–250 sweat ducts, the same area of cattle skin contains ~2000 hair follicles (16, 17) with their associated sweat and sebaceous glands and ducts; sheep skins can contain up to 10,000 follicles with their associated ducts (20, 21).

The resistance to penetrating molecules, R, offered by multilayered human skin that consists of a stratum corneum (with resistance R_S), a viable epidermis (with resistance R_E), and a dermis (with resistance R_D) is given by (10):

$$R = R_S + R_E + R_D \qquad \text{(Eq. 2)}$$

Equation 2 can be restated as:

$$R = \frac{1}{k_p} = \frac{\delta S}{D_S K_S} + \frac{\delta E}{D_E K_E} + \frac{\delta D}{D_D K_D} \qquad \text{(Eq. 3)}$$

where:

k_p = permeability constant of skin
δ_i = thickness of ith layer
D_i = diffusion constant of penetrant in ith layer
K_i = ith layer–vehicle partition coefficient of penetrant

Studies on the penetration of excised human skin by small to medium molecular weight molecules (10, 12) revealed that the first terms in Eqs. 2 and 3 describe the process. Thus, for a homologous series of straight-chain aliphatic alcohols with one to eight carbon atoms, the stratum corneum was the rate-determining barrier. Variations in the thickness or other properties of the viable epidermis or dermis had no effect on the rate of penetration. A similar situation applied to the penetration of levamisole (IV) through excised human skin specimens

Table VI—Relative Density of Sweat Glands and Area of Secretory Surface per Square Centimeter of Skin for Ayrshire Cattle [a]

	Density of Sweat Glands, cm^{-2}	Area of Secretory Surface per Square Centimeter of Skin, cm^{-2}
Above average	Neck ventral, neck lateral, axilla	Neck ventral, axilla, upper hindleg
Below average	Back sacral, gluteus, lower hindleg	Forehead, back sacral, lower foreleg, gluteus, lower hindleg

[a] From Ref. 16.

whose thicknesses varied from 1.3 to 0.8 mm (13). The average thickness of the human stratum corneum is 0.001 mm (11); therefore, all skin specimens in this experiment contained a uniform thickness of stratum corneum and varying thicknesses of viable epidermis and dermis.

However, the rate of penetration of IV through excised cattle (13) and sheep (71) skins with thicknesses that varied from 1.7 to 0.7 mm was dependent on the total skin thickness. The stratum corneum of cattle (15) and sheep (14) skin was reported to be 3.09 and 3.14×10^{-2} mm, respectively, so all skin samples examined in these studies had their stratum corneums intact.

This evidence suggests that the rate-determining barrier to cattle and sheep skin penetration is not the stratum corneum but the whole skin. In the case of sheep skin penetration, it is known that the entire stratum corneum is penetrated by an emulsion of sebum and sweat (14). Hence, in this case, it may be argued that molecules indeed penetrate the stratum corneum *via* lipid regions in which they have similar diffusivity to that in the medium surrounding the cells in the epidermis and dermis. However, it was noted that the lower one-third of the stratum corneum of cattle skin (~0.01 mm) is not permeated by the emulsion (15), thus presenting a similar barrier to penetration as that of the human stratum corneum.

Another possible explanation is that levamisole passes rapidly through the stratum corneum and its rate of clearance into the lower skin layers is rate determining. Higuchi (77) estimated that very lipophilic molecules (*i.e.*, those with activity coefficients of 10^3 or 10^5) in infinitely dilute solutions in water would encounter rate-determining clearance from the stratum corneum into the more aqueous

Table VII—Summary of Dermal Drug Delivery Studies

Study	Animal	Drugs	Purpose	Comments	Reference
1	Cattle	I, II, VIII, IX, X, XI, XII, XXI, XXII	Pour-on against southern cattle tick	VIII and IX superior	78
2	Cattle	VII	Bioavailability		79
3	Cattle	I	Pour-on against GI parasites	Poor against abomasal parasites; erratic against intestinal parasites	80
4	Cattle	I	Pour-on versus spray, intramuscular and intraperitoneal injections against *Hypoderma* spp.	All dosage forms >93% effective	84
5	Cattle	XIX	Pour-on against cattle lice	62.3–97.8% effective	88
6	Cattle	IX	Pour-on against cattle lice	Effective	89
7	Cattle	VIII, IX, XIV, XV	Pour-ons against long-nosed sucking lice	Most effective for a short term	6
8	Cattle	XIII	Pour-on versus intramuscular injection against cattle grub	Equivalent	90
9	Cattle	I	Pour-on against nematodes	Erratic results except against *H. placei*	91
10	Cattle	44 compounds	Screen for systemic activity	Variable	92
11	Cattle	I	Spray *versus* pour-on against cattle grub	Pour-on superior	1
12	Cattle	XIII, XIV, XIX	Comparison of XIV with XIII and XIX against cattle lice	XIV at 4 mg/kg equivalent to XIII at 16.5 mg/kg, XIX at 4.5 mg/kg	93
13	Cattle	II	Comparison of pour-on and intramuscular injection *via* spray	Pour-on and intramuscular injection superior	94
14	Cattle	I	Pour-on anthelmintic	Effective against five nematodes, ineffective against four nematodes	95
15	Cattle	II	Bioavailability of spray	Little absorbed	83
16	Cattle, sheep	VII	Bioavailability	—	96
17	Cattle	I, II, VI, VII, VIII, XVIII, *etc.*	Comparison of pour-ons against cattle grub	Variable results	97
18	Cattle	I, VI, VIII	Spray and pour-on of VIII *versus* sprays of I and VI against cattle grub	All exceed LD_{90}	98
19	Cattle	I, II, VI	Pour-ons *versus* dusts and sprays against horn flies	Dusts and pour-ons superior to sprays	99
20	Cattle, sheep	10 compounds	Saturated sprays and pour-ons against cattle ticks	Maximum protection: sheep, 3 weeks, cattle, 1 day	100
21	Cattle	XX	Bioavailability from 1% suspension	No absorption	101
22	Cattle	XXIII	Pour-on for estrus synchronization	Equivalent to oral	102
23	Cattle	IV	Toxicity study	All complications corrected in 1–4 weeks	103
24	Cattle	IV	Anthelmintic trial of pour-on	Variable results but comparable to orthodox methods	104
25	Cattle	IV	Spot-on against round worms	Effective	105
26	Cattle	IV	Spot-on against lung and GI worms	Effective	85
27	Cattle	IV	Pour-on against nematodes	Equivalent to oral and subcutaneous dosage forms	80
28	Cattle, sheep	IV	Pour-on against nematodes	Effective	86
29	Sheep, goats	XIX	Pour-on against lice and keds	Effective against goats, hairy and wool/hair sheep; less effective against wool sheep	[a]
30	Sheep	XXIII	Pour-on against *Fasciola hepatica*	Worms in liver reduced 99–100% in 3 weeks	87

[a] H. J. J. Terblanche, Bayer, South Africa (Pty.) Ltd. personal communication.

skin layers (*i.e.*, epidermis and dermis). This situation, which is essentially the same as one where the last two terms in Eqs. 2 and 3 predominate, was discussed by other investigators (10, 12, 39). However, it is unlikely that IV, which has a partition coefficient of only 5.2 between water and toluene (70), is sufficiently lipophilic to experience rate-determining clearance from the stratum corneum. The data on human skin penetration (13), where the stratum corneum was established to be the rate-determining barrier, also suggest that IV is not highly lipophilic. Thus, these *in vitro* results support the conclusion that IV penetrates cattle skin *via* skin appendages.

Further confirmation comes from the value calculated for the diffusion constants for IV in cattle skin (2.1×10^{-5} cm^2/min) (13) and sheep skin (2.5×10^{-5} cm^2/min) (71). These values are much closer to those reported for water diffusing through human hair follicles ($0.3–1.2 \times 10^{-5}$ cm^2/min) (10) or sweat ducts ($6.0–12.0 \times 10^{-5}$ cm^2/min) (10) than to the value calculated for water ($3–4 \times 10^{-8}$ cm^2/min) (10) or IV (1.9×10^{-9} cm^2/min) (13) penetrating through the stratum corneum.

Additional support comes from considering the effect that dimethyl sulfoxide has on penetration through cattle skin (71), sheep skin (70), and human skin (11). The dramatic accelerating effect that dimethyl sulfoxide has on the penetration of many molecules through human skin is in marked contrast to its effect on levamisole penetration through excised cattle and sheep skins where it slightly decreased penetration rates relative to those from aqueous solvents. Because this accelerating effect has been ascribed to dimethyl sulfoxide's ability to alter temporarily the structure of the stratum corneum, it can be concluded that drugs do not penetrate cattle and sheep skins by this route. The decrease in penetration rate through cattle and sheep skins caused by dimethyl sulfoxide is believed to result from its reduction of the thermodynamic activity of the drug in the applied formulation (70).

If the transappendagial route is the major means of bulk transport through cattle and sheep skins, it does not necessarily follow that the total skin thickness, depth of follicle, or length of sweat or sebaceous ducts would greatly influence penetration rates in live animals as they apparently do in excised skin. The hair follicles and ducts are richly supplied by blood, and drug molecules are likely to pass rapidly through the capillary walls and be carried away by the blood as they diffuse through the appendages.

If transappendagial penetration predominates in cattle and sheep skins and transcellular penetration predominates in human skin, some procedures that have been adopted to promote human skin penetration will not necessarily be effective in promoting cattle and sheep skin penetration. The procedures in question were well reviewed (10–12, 76), and they include hydration of the skin and the use of stratum corneum modifiers such as dimethyl sulfoxide or surfactants.

However, other procedures such as maximization of the concentration gradient or, more accurately, the thermodynamic activity gradient of a drug across the rate-determining barrier and of the rate-determining barrier–vehicle partition coefficient will be equally important in promoting cattle and sheep skin penetration as in promoting any Fickian diffusion process (77). Thus, if molecules diffuse through the skin appendages and these present a homogeneous barrier, the flux J is given by:

$$J = \frac{D_A K_A}{\delta_A} \Delta C_S = k_{P,A} \Delta C_S \qquad \text{(Eq. 4)}$$

where:

D_A = diffusion constant of penetrant molecules in appendages
K_A = appendage–vehicle partition coefficient of penetrant
δ_A = thickness of appendages that must be traversed
ΔC_S = external concentration difference
$k_{P,A}$ = permeability constant of penetrant molecules in appendages

Poulsen (76) and others (10–12) discussed how an understanding of the density of the stratum corneum (because $D \propto 1/\text{viscosity}$) and its solvent and physicochemical properties have been utilized to control dermal delivery to humans. Similar approaches are necessary to promote dermal delivery to cattle and sheep but will necessitate an understanding of the viscosity and physicochemical properties of the appendagial pathways.

Anatomical and physiological studies of cattle and sheep skins, discussed earlier, indicate that the nature of the skin emulsion formed from sebum and sweat holds the clue to skin permeability. The emulsion forms in the infundibula of the follicles and, in oozing through follicle pores and through all (in sheep) or the upper layers (in cattle) of the stratum corneum, results in the deposition of emulsified sebum around the emerging fibers and, to a greater (in sheep) or lesser (in cattle) extent, on the wool and hair fibers. This emulsion probably is the medium through which molecules must pass to reach a point where they can diffuse through capillaries into the blood.

If this conclusion is correct, seasonal changes in the composition of the emulsion that result from previously discussed seasonal changes in sweat and sebum output are the reason why drugs appear to penetrate cattle skin faster in summer than in winter. *In vitro* studies on penetration of levamisole (IV) through excised cattle skin revealed that the drug penetrated 10 times faster through skin harvested in summer than through skin harvested in winter (71). A similar conclusion was drawn from measurements of blood levels of IV in cattle following application of trial pour-on formulations in summer and winter (71). Another possible example of reduced skin permeability in winter as compared to summer was the report (78) that a pour-on phosmet (VIII) at 40 mg/kg gave excellent control of Southern Cattle Tick when applied to cattle in Australia in March (early fall) or September or October (spring) but poor control in May (winter).

Preliminary results indicate that the penetration barrier (*i.e.*, in all probability, the emulsion) is a relatively polar solvent for penetrant molecules. Thus, studies of the penetration of IV through excised cattle skin (71) indicated that the partition coefficient of IV from aqueous solutions where it existed primarily as a neutral molecule was 0.84. The substitution of water by less polar solvents (*i.e.*, better solvents for IV) resulted in an apparent reduction in the skin–solvent partition coefficients. Dedek (72) concluded that polar organophosphorus compounds (*i.e.*, those with

relatively high water solubility) penetrate cattle skin most rapidly from solvents with low polarity, whereas nonpolar Compounds penetrate most rapidly from polar solvents.

Before a definitive correlation of these results with the solvent properties of the barrier can be made, it must be established (77) that diffusion in the barrier rather than clearance from it into an aqueous environment is rate determining for the organophosphorus compounds. Nevertheless, Dedek and coworkers (72–74) set out useful guidelines for formulation of topical drug delivery systems. The solubilities of some insecticides are given in Table I. Values of 738 compounds are in Ref. 75.

An additional problem arises if diffusion through the emulsion is the preferred pathway for drug absorption. Hence, if a dosage form is designed so that the drug has a favorable emulsion–vehicle partition coefficient, the drug will also have a strong tendency to diffuse up the emulsion coat on the hair and wool fibers in competition with its diffusion into the lower skin layers. This phenomenon could reduce the rate of skin penetration, but it could also lead to relatively sustained release of a drug over an extended period because the surface emulsion would act as a drug depot.

It must be reemphasized that the foregoing discussion is highly speculative and is included to stimulate experimental approaches towards elucidation of absorption mechanisms.

POUR-ON AND SPOT-ON FORMULATIONS

Table VII lists some trials performed with pour-on or spot-on formulations on cattle and sheep. While there is a substantial body of evidence that topical drug delivery can be effective, the studies provided little information on the absorption mechanism and thus little information that could be used as a basis for designing future topical drug delivery systems. Some of the reasons for this sorry state of affairs are:

1. Failure to report the solvents used in trials. All but three of the reports tabulated in Table VII failed to mention the solvents used. Words such as "a suitable solvent," "a rapidly evaporating solvent," "a slowly evaporating solvent," or "an emulsifiable solvent" were used. Therefore, it is not possible to ascertain whether the failure of certain trials arose because the partition coefficient between the penetration barrier and the solvent or vehicle was so unfavorable that the rate and extent of absorption (*i.e.*, the bioavailability) were unacceptably low or because the drug was not active systemically. Furthermore, it is known that many organic solvents damage skin. This effect could increase drug bioavailability to a point where it gave an acceptable therapeutic response but produced unacceptable inflammation or lesions due to bacterial proliferation.

Without knowing the solvent used, it is also impossible to separate skin damage caused by residual drug from that possibly caused by the solvent. An example of the problems caused by failure to specify the solvents used in the formulations is provided by a report (78) of a comparison of nine pour-on formulations of organophosphorus insecticides against the Southern Cattle Tick in Australian Illawarra Shorthorns (Study 1 in Table VII). Because the solvents were not mentioned, it was impossible to evaluate why phosmet (VIII) at 40 mg/kg and chlorpyrifos (IX) at 60 mg/kg gave better tick control than the other seven products or why IX caused severe hair loss and skin burns while the VIII formulation did not.

2. Failure to restrain animals so that they cannot lick themselves or other animals. Drugs applied to the skin of animals can enter the systemic circulation by transdermal absorption or orally if the animals ingest the drug by licking. In many cases, drugs were applied to animals in such a way that they could not lick them off themselves (*e.g.*, drugs applied to cattle on the dorsal midline extending backward from the withers), but frequently no steps were taken to prevent the animals from licking drug from other animals.

The danger of this latter event leading to erroneous results is illustrated by a study (79) (Study 2 in Table VII) in which ronnel (VII) was applied on the dorsal midline of cattle from the hip forward and drug concentrations in the blood and other tissues were monitored as a function of time. The animals were yoked and their tails were tied for the first 10 days to prevent any oral ingestion; they were then allowed to run free. The blood levels of VII passed through a maximum after 36 hr and then fell away exponentially until about Day 11 when there was a dramatic increase in blood levels, eventually reaching a blood concentration that was 50% higher than the initial maximum. This phenomenon resulted from oral ingestion of drug after the animals were allowed to run free.

No difference in efficiency of a levamisole pour-on against nematodes in cattle was observed between restrained and free cattle (80). This result could arise because levamisole, as would be expected from the *in vitro* skin permeability studies (13), is very rapidly absorbed.

While it is appreciated that commercially useful pour-on formulations will be applied to free running animals, care must be exercised in judging the effectiveness of a formulation on the basis of trials where oral ingestion of topically applied drugs is possible.

3. The use of imprecise end-points to evaluate products. The ideal end-point for the evaluation of a drug delivery system is the therapeutic or pharmacological response that it is intended to elicit. However, as illustrated by Poole and Dooley (81) (Study 3 in Table VII), great care must be exercised in selecting the correct end-point. These authors discussed the effectiveness of a crufomate pour-on as an anthelmintic against GI parasites in cattle on the basis of both the egg count per gram of feces and the helminth counts at necropsy. The latter parameter indicated that the dosage form had no anthelmintic effect on abomasal helminths and had eratic efficacy on intestinal parasites. This finding was in contrast to an earlier report (82) that concluded, on the basis of egg counts per gram of feces only, that the treatment was effective against a variety of GI parasites in cattle. The authors (81) pointed out that ova in the feces indicated only the presence of an ovulating female helminth and should be used only as a diagnostic tool.

Because it is wasteful and often not possible to carry out necropsies to evaluate anthelmintic activities of pour-on formulations, it seems highly desirable to obtain blood level *versus* time profiles for drugs following dermal application and to use this information as a basis for developing new formulations.

These points illustrate the need to use caution in interpreting currently available reports on the effectiveness of pour-on formulations.

Retention of Drugs on Skin—Some reports indicated that a number of topically applied drugs persist on the skin or coat of animals for long periods and that only a fraction of the applied dose is absorbed (poor bioavailability). For example, the application of a 4% formulation of ^{32}P-labeled VII in paraffin oil to cattle (Study 2 in Table VII) resulted in the appearance in the blood of an organosoluble fraction (undegraded drug and an oxygen analog) and water-soluble breakdown products (79). The maximum concentration of the organosoluble fraction was reached in 48 hr in restrained animals and had fallen to zero by Day 9. However, an appreciable amount of radioactivity was still present on the skin and hair after 10 weeks.

Although only very small amounts of ^{32}P-labeled II were absorbed by cattle following the use of a dilute spray solution (in 20% xylene–octoxynol[2]), high levels of unchanged drug could still be detected on the skin after several weeks (83) (Study 4 in Table VII). Similarly, high levels of I were present on the skin of cattle 30 days after treatment with both a spray and pour-on made by diluting an emulsifiable concentrate of drug with water (84) (Study 5 in Table VII). These phenomena presumably result from chemical interactions of the drugs with the hair fibers or the skin proteins as discussed previously.

The ways in which these phenomena are likely to vary with the chemical properties of the drug are illustrated by reports that the bioavailability of IV from a pour-on (80) and spot-on (85) formulation was as good as or better than that from an oral formulation (Studies 6 and 7 in Table VII).

Solvent Systems and Methods of Application—Although many investigators have failed to report the exact solvent systems used in individual studies, there are frequent references to generic solvents. Pour-on formulations often consist of a suspension of a wettable powder in water or light mineral oils. Manufacturers do not usually reveal the formulation of the wettable powders, but they are generally composed of a wetting agent, a dispersing agent, and a clay or other inert carrier together with the drug. The wettable powder is employed to maximize the rate of dissolution of solid drug following its application to the skin.

Suspensions are also sometimes prepared in light liquid paraffin or other light mineral oils or oil-based water-immiscible drug solutions.

Light mineral oils are usually poor solvents for drugs, but they are often included in suspensions or solutions to hold the drug on the animal's skin. Some other solvent systems that have been described are 10% dimethyl sulfoxide-aromatic hydrocarbon (86), 10% cyclohexanol–aromatic hydrocarbon (86), dimethyl sulfoxide–amyl alcohol (1:4) (87), and various isopropanol-based solvents that contain light mineral oils, surfactants, or aromatic and nonaromatic solvents.

Water-insoluble compounds are often formulated as an emulsifiable concentrate. The emulsifiable concentrate contains one or more surfactant and produces an emulsion or micellar solution, with the water-insoluble drug in the nonaqueous phase, when mixed with water.

Various methods have been described for applying topical drug delivery systems. The simplest and most commonly used method is to pour the drug solution from a beaker or similar vessel onto the skin of the animal. Application of drugs to sheep skin has been achieved by parting the wool and dispensing the formulation from a needleless syringe (86).

One study (104) reported that the area of cattle skin to which IV had been applied was kept damp for several days. However, the effect of this procedure on penetration rates was not recorded.

One report (96) highlighted the fact that VII was absorbed more effectively from shorn than from unshorn areas of sheep skin.

FUTURE WORK

There is an urgent need to establish firmly whether a relationship exists between the permeability of excised cattle and sheep skins to drug formulations in *in vitro* experiments and the permeability of the skins in live animals. Such relationships have been established (10, 12) for human skin penetration, and this fact has led to the production of a vast amount of quantitative information about the process.

If no direct relationship is found, it will suggest that such factors as the rate of production of sebum or sweat, the rate of blood flow, and even the rate at which keratinized epithelial cells are shed by the skin of live animals may be involved in the dermal drug delivery process. If this is the case, experiments must be carried out on live animals under various conditions to permit a rational approach to controlled dermal delivery. These experiments must be performed under conditions where applied drugs cannot be ingested and where their chance of being removed by rain or other environmental forces is minimized.

If the permeability of excised skin is directly related to *in vivo* behavior, the task of characterizing the absorption mechanism will be easier. The experimental methods already developed to study human skin penetration (13) can be applied to obtain quantitative information about the effects of breed, season, climate, age, nutritional status, and formulation on the transdermal delivery process.

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