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Note

The effect of freezing on cattle skin permeability

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Summary

The effect of freezing temperature and storage time on the permeation characteristics of cattle skin as measured by in vitro permeation studies of water, uracil, hydrocortisone, abamectin, and ivermectin was investigated. The effect of freezing at −20 and −70°C on the in vitro permeation of all compounds except abamectin was significant at all storage periods from 2 weeks to 6 months. There appear to be no general trends in the changes of permeation characteristics of the compounds studied due to freezing of the cattle skin.

Percutaneous absorption of compounds across excised human and animal skin has been investigated extensively (Barry, 1983). A review of the literature shows that the skin used in many of these studies had been stored under various freezing conditions until it was needed (Bronaugh and Maibach, 1986; Scott et al., 1990). Although the effect of storage conditions, e.g., freezing time and temperature, has been shown to significantly diminish the metabolic activity of the skin for some compounds, the influence on the barrier properties of the skin appears to be still disputed (Franz, 1975; Swarbrick et al., 1982; Higo et al., 1992). This is mainly due to a lack of comparable studies that address the effect of freezing on skin permeation as a function of species, freezing temperature, and in particular the compounds studied. For example, Harrison et al. (1984) measured the permeation of water across human skin and found no significant differences between fresh and frozen samples (−20°C) over a period of 466 days, whereas Hawkins and Reifenrath (1984) observed an increase in the permeation rate of *N,N*-diethyl-*m*-toluamide across pig skin stored for a period of 6 weeks at −80°C.

In view of such discrepancies and differences, this study examines the effect of freezing temperature and storage time on the barrier properties of cattle skin as measured by the permeation profiles of compounds of increasing molecular size and hydrophobicity; water, uracil, and hydrocortisone, along with abamectin and ivermectin (Table 1). The latter two are antiparasitic agents of the avermectin family with wide-spectrum activity against both endoparasites and ectoparasites in cattle. Cattle skin was used specifically

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TABLE 1

The physical chemical properties of the compounds

Compound	Mol. Wt (g/mol)	Solubility in propylene glycol (mg/ml)	Log P [a]
Uracil	113.1	1.96	−1.056
Hydrocortisone [b]	362.5	12.7	0.139
Abamectin	870.3	7.05	2.103
Ivermectin	872.3	225.9	2.647

[a] Calculated logarithm of octanol/water partition coefficient (Advanced Modeling Facility, v. 1.12, Merck Research Labs).
[b] From Merck Index, 11th Edn, 1989.

because of the large amount of skin that can be excised from a single location of only one animal to conduct a large-scale systematic study.

Uracil and hydrocortisone (98%) were obtained from Sigma Chemical Co. and used as received. Abamectin (avermectin B_1) and ivermectin (22,23-dihydroavermectin B_1) were supplied by Merck Sharp & Dohme Quimica de Puerto Rico (Barceloneta, Puerto Rico). Tritiated water (NEN® Research Products, Boston, MA) at a concentration of 2.3 μCi/ml was used as the donor solution in water permeation studies. Saturated solutions of uracil, hydrocortisone, abamectin, and ivermectin in propylene glycol were used in permeation studies (Table 1). [5,6-^{3}H]Uracil and [1,2-^{3}H(N)]hydrocortisone (NEN® Research Products) and [22,23-^{3}H]dihydroavermectin B_{1a} and [5-^{3}H]avermectin B_{1a} were added to their respective saturated solutions to give a final concentration of 5.3 μCi/ml.

Skin samples were excised from Holstein dairy cattle. The hair was clipped as close as possible to the skin shortly after killing and skin samples were removed from the dorsal thoraco-lumbar region. These samples were either used within 1 h (fresh skin) or wrapped in plastic bags and placed in freezers maintained at either -20 ± 1 or -70 ± 2°C for later studies at which time they were thawed at room temperature prior to use. The thickness of the dermatomed skin samples was 4.8 ± 0.2 mm. Water permeation experiments were performed with skin obtained from one cow and all other experiments were carried out with skin from another cow.

Modified Franz diffusion cells (Model, FDC-400, Crown Glass Co., Somerville, NJ) were used. The receptor compartment had a volume of 12.2 ml. The diffusional area was 1.77 cm^2. The volume of the vehicles in the donor chamber was 1.0 ml. A thermostated water bath circulated water at 39 ± 1°C through the diffusion cell jackets in order to regulate the temperature to approximate that of cattle skin. At each sampling time 200 μl of the receptor phase was withdrawn for analysis and replaced with equal volume of fresh receptor phase solution. The receptor compartment was filled with 25% glycerol formal in water to provide sink conditions for the permeation of drugs. The presence of 25% glycerol formal in the receptor phase does not affect the cattle skin barrier properties (data not shown).

Permeability coefficients were calculated from the linear portion of the permeation profiles to determine whether the permeation rates for the stored skin samples were different from that for the fresh skin. Permeation profiles of water across cattle skin at various storage times at −20°C are shown in Fig. 1. The permeability coefficient, K_p, was calculated using the equation:

$$K_p = J/(A\Delta C)$$

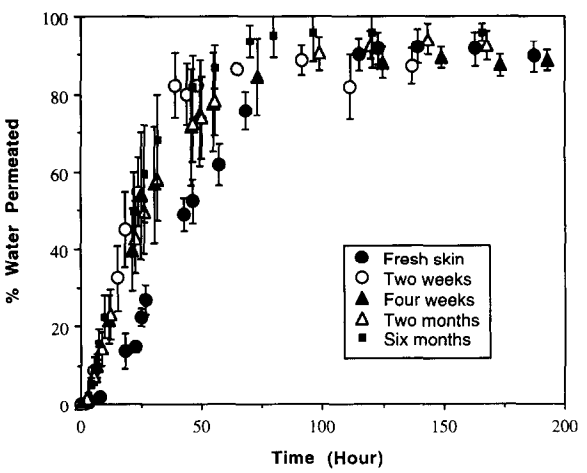

Fig. 1. Permeation profiles of water across cattle skin stored at −20°C from 2 weeks to 6 months along with that of fresh skin ($n = 5$).

TABLE 2

Permeability coefficients for water across the cattle skin (errors are quoted as standard errors, n = 5)

Storage time at −20°C	Permeability coefficient ($\times 10^2$) (cm/h)
Fresh	0.75 ± 0.02
2 weeks	1.25 ± 0.04
4 weeks	1.18 ± 0.06
2 months	1.12 ± 0.05
6 months	1.52 ± 0.03

where J is the steady-state flux (slope of the linear portion), ΔC denotes the concentration gradient across the skin which was taken as the donor concentration since the receptor drug concentration never exceeded 2% of the donor in all cases, and A is the area of diffusion. The permeability coefficients for the water permeation data are given in Table 2. The permeability coefficient for the fresh skin was significantly lower ($p < 0.05$) than those for skin frozen at −20°C at all storage periods.

The permeability coefficients for uracil, hydrocortisone, abamectin, and ivermectin are listed in Table 3. For uracil, the permeability coefficient of samples stored at −20°C for 2 weeks was less than that of fresh skin samples, however, the permeability coefficient values increased for 6 week and 6 month storage periods. Furthermore, the permeability coefficients more than doubled for the samples stored for 6 months, independent of the storage temperature when compared to fresh skin. There appeared to be a gradual increase in the permeability coefficients of hydrocortisone for samples stored at −20°C from 2 weeks to 6 months. There was no significant difference in the permeability coefficients of hydrocortisone stored for 6 months at either −20 or −70°C.

There were no significant changes in the values for the permeability coefficients of abamectin between the fresh and frozen samples stored at −20 and −70°C. The permeability coefficient for ivermectin across fresh skin was significantly different ($p < 0.05$) from those for skin stored at either −20 or −70°C. Furthermore, skin samples stored at −20 and −70°C were not significantly different from each other with regards to permeation of ivermectin. The difference in the permeabilities of ivermectin and abamectin, the latter having a lower permeability coefficient in cattle skin which has been stored frozen, is not fully understood, since these compounds have basically the same chemical structure and molecular weight and vary only in the hydrogenation of the C-22–C-23 bond.

There were no significant metabolites or degradation products of any of the compounds studied in the receptor phases of the cattle skin samples frozen for at least 1 week as determined by high-pressure liquid chromatography. The increased permeation observed with frozen samples for water, hydrocortisone, and ivermectin along with the absence of any significant degradation products suggests structural changes in the skin, e.g., stratum corneum which has been proposed as the rate-limiting barrier to penetration (Barry, 1983). Upon freezing the skin, water crystallises and can damage the intracellular lipid matrix or the keratinised cells such that both the so-called 'polar' and 'lipid' pathways are affected and the

TABLE 3

Permeability coefficients of selected compounds across cattle skin stored under various conditions (errors are quoted as standard errors, n = 5)

Storage condition	Permeability coefficient ($\times 10^5$) (cm/h)			
	Uracil	Hydrocortisone	Abamectin	Ivermectin
Fresh	45.3 ± 0.3	0.43 ± 0.01	0.28 ± 0.03	0.29 ± 0.02
2 weeks (−20°C)	29.3 ± 6.8	1.04 ± 0.06	0.28 ± 0.02	0.45 ± 0.05
6 weeks (−20°C)	53.4 ± 7.1	1.90 ± 0.10	0.26 ± 0.04	0.51 ± 0.03
6 months (−20°C)	134.3 ± 11.5	3.38 ± 0.24	0.27 ± 0.02	0.40 ± 0.04
6 months (−70°C)	129.3 ± 7.7	3.76 ± 0.18	0.23 ± 0.02	0.44 ± 0.03

overall permeation of the skin is increased (Swarbrick et al., 1982; Flynn, 1983). Such structural changes in cattle skin were not detected by routine histological studies. The permeation may also be affected by the loss of metabolic activity of the skin upon freezing; this may explain the initial decrease in the permeability of uracil upon freezing.

It is reasonable to assume that samples of skin excised from animals immediately after killing retain most of the characteristics of the skin in vivo. Unfortunately, there are significant practical problems associated with this method of experimentation, in particular the dependence of scheduling experiments on the availability of sample, supply of which can be erratic. Therefore, it is desirable to freeze the skin under storage conditions such that it closely resembles fresh skin. The results of this study suggest that this goal is not fully attainable in cattle skin. It is clear that once the skin is frozen, its permeation characteristics are irreversibly altered. Hence, no quantitative measurements can be performed with frozen skin to reliably predict performance in vivo. Nevertheless, frozen cattle skin provides valuable qualitative information with regards to rank of order in permeability of various compounds since the permeation profiles of fresh and frozen samples are very similar.

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