thickness will increase with decrease in film thickness. Assuming that the surface layer has a greater Young's modulus than the bulk film, the Young's modulus of the bulk film will increase with decrease in film thickness, this is the observed phenomenon.

It is possible, however, that the decrease in strength with film thickness is due to the weakening effect of minor imperfections being proportionately greater in the thinner films. These imperfections could arise as a consequence of the method of preparing the test samples, by cutting from a sheet, or from residual internal stresses forming during the drying phase.

Very thin films formed from polymeric binders, such as will be present in tablet granules, are much weaker and more brittle than the films that have been tested previously. However, methylcellulose films are stronger than maize starch films at all thicknesses despite the differences in slopes of the regression lines.

References

Abdel-Aziz, S. A. M., Anderson, W., Armstrong, P. A. M. (1975) J. Appl. Polym. Sci. 19: 1181–1192
Aulton, M. E. (1982) Int. J. Pharm. Tech. Prod. Mfr. 3(1): 9–16
Bacon, R., Smith, W. H. (1965) in: Proceedings of the Second Conference on Industrial Carbon and Graphite, Society of Chem. Industry, London. pp 203–213
Healey, J. N. C., Rubinstein, M. H., Walters, V. (1974) J. Pharm. Pharmacol. 24 (suppl): 41P–46P
Jenkins, C. M., Kawamura, K. (1976) in: Polymeric Carbons, Cambridge University Press, p. 119
Jones, P.F., Duncan, R. G. (1971) J. Mater. Sci. 6: 289–293
Merck Index (1968) 8th Edition, Merck and Co., Rahway, USA
Reading, S., Spring, M. S. (1984) J. Pharm. Pharmacol. 36: 421–426
Schott, H. (1970) J. Pharm. Sci. 59: 1492–1496
Winston, P. W., Bates, D. E. (1960) Ecology 41: 232–237

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The permeability of grafted human transplant skin in athymic mice

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Abstract—Human skin has been transplanted onto athymic mice and its permeability properties assessed to see if this in-vivo model would be of benefit in predicting accurately absorption of drugs or toxic chemicals through human skin. The permeability properties of the skin alone, and grafted and athymic mouse skin were assessed by measuring in-vitro absorption of tritiated water and a permanently charged cationic penetrant, paraquat. The grafted skin and athymic mouse skin had similar permeability to the tritiated water. However, the grafted skin was less permeable to paraquat but was more permeable to it than normal human skin, indicating that although histologically, the transplanted skin appeared normal, its barrier properties were impaired. The model was not, therefore, useful for assessing human percutaneous absorption.

Industrial chemicals and pesticides may accidently come into contact with human skin and drugs may be deliberately applied for beneficial effects. Sufficient quantities can be absorbed through the skin to cause toxic reactions (Davies et al 1979). Assessment of percutaneous absorption as a contribution to predicting the effect of substances in man, requires the use of animal studies. However, the structure of mammalian epidermis varies from species to species. There are microscopically obvious differences such as presence or absence of sweat glands, number of hair follicles and number of cell layers in the epidermis. Other differences, such as the amount of lipid present at different body sites may be less obvious but have also been reported (Elias et al 1980). Differences in the number of cell layers and biochemical composition are seen in the stratum corneum, the outer layer of corneocytes, which forms the primary diffusion barrier (Blank 1965). Although there is no perfect animal model for human skin permeability (Scott et al 1986a), in-vitro percutaneous absorption techniques have been developed which can predict in-vivo absorption (Scott et al 1986b). We have used such an in-vitro technique to compare the permeability properties of human and laboratory animal skins (Dugard et al 1984). The in-vitro techniques developed to date, however, have not yet replaced in-vivo methods completely and there is scope for further refinements to existing protocols.

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Recently, human cadaver and biopsy skin, transplanted onto immune-suppressed mice, has been reported to maintain a morphology similar to normal human skin (Kruger & Briggaman 1982) and to retain human microflora not found normally on the host mouse (Kearney et al 1982). If such grafted human skin is to be used to predict potential absorption in man, the permeability properties of the transplanted skin must be comparable with normal human skin. To investigate this we have measured the absorption of two test penetrants (water and paraquat ion), through the athymic mouse and grafted human skin. We have previously used these penetrants as markers for abnormal permeability properties and species differences (Dugard et al 1984; Scott et al 1986a).

Materials and methods

Chemicals. Tritiated water was obtained from the Radiochemical Centre, Amersham, UK, and diluted with distilled water to a final activity of 5 μCi mL^{-1}. Paraquat dichloride was obtained from the Radiochemical Laboratory, ICI plc, Petrochemicals Division, Billingham, UK, and added to a 1000 mg mL^{-1} solution of paraquat dichloride in distilled water to a final activity of 8 μCi mL^{-1}.

Skin graft. Cadaver skin was obtained from a hospital mortuary, between 24 and 72 h after death. Thoracic skin was transplanted onto athymic mice using published methods (Kearney et al 1982) and then, to prevent infection, the mice were housed in filter top cages at a temperature of 28–30°C and relative humidity of greater than 60%. The skin grafts were maintained on these mice for up to 6 months.

Permeability assessment. The procedure followed has previously been reported by Dugard et al (1984). Essentially, whole skin (epidermis plus dermis) membranes were mounted in glass diffusion cells. On Day 1 of the assessment the permeability of

the skin membranes (human, grafted human and athymic mouse) to tritiated water was measured. At the end of the experiment (6 h) the donor and receptor chambers were emptied. Saline (0·9%NaCl), 0·5 mL, was placed in the receptor chamber (typical volume 4·5 mL) and the membranes left to hydrate naturally overnight, under ambient laboratory conditions. On Day 2 of the experiment, the permeability of the membranes to paraquat was assessed.

Results and discussion

The grafting technique macroscopically appeared successful, in that viable grafts, similar to pregrafted human skin, were obtained. Visually, the grafts appeared healthy and raised above the thinner mouse skin. Within 3 to 4 weeks after transplanting, hair regeneration was seen in grafts (of predomoninantly male origin).

The histopathological findings showed that the hair follicles, sebaceous glands and sweat glands were retained, epidermal and dermal structure was similar to typical human skin, and the grafted skin was well vascularized. Increased melanin pigment production, mild hyperkeratinization, slight epidermal necrosis and leucocyte infiltration were seen in some skin samples, but were believed to be a feature of normal peri-mortem changes.

The permeability results, expressed as a calculated (absorption rate divided by applied penetrant concentration) permeability constant, Kp (units, cm h^{-1}) are presented in Table 1. The permeability of the human grafted skin and mouse skin to tritiated water was similar. We have already shown (Scott et al 1986a) that with this small, polar molecule, normal human and some common laboratory animal skins have similar permeability. However, as in that previous study, the mouse and grafted skins were significantly more permeable to tritiated water, than ungrafted human skin ($P<0·001$, Student's t-test). With the permanently charged di-cation, paraquat, the human grafted skin was significantly less permeable than the athymic mouse host skin ($P<0·002$, Student's t-test) and significantly more permeable than the cadaver skin ($P<0·001$, Student's t-text). However, the human grafted skin had a permeability to paraquat of the same magnitude as we had previously found with the skins of other animal species in-vitro even though normal human skin is 40–50 times less permeable than animal skin (Scott et al 1986a).

Table 1. The in-vitro permeability of human grafted skin alone and when on athymic mice, and of athymic mouse skin. Permeability values are presented as calculated permeability constants (Kp, units $\times 10^{-3}$cm h^{-1}, s.e.m.; n = number of determinations).

Substance	Human skin	Grafted human	Athymic mouse
Tritiated water	0·93 (0·14; n = 7)	2·43 (1·89; n = 7)	2·34 (1·71; n = 8)
Paraquat	0·007 (0·002; n = 7)	0·82 (0·25; n = 5)	4·81 (2·00; n = 5)

As we were unable to prepare intact epidermal membranes from the available grafted human skin and host mouse skin, whole (epidermis plus dermis) was used. In the in-vitro system the aqueous dermis can act as an artificial barrier to the penetration of non-polar molecules (Scheuplein & Blank 1973; Scott et al 1986c). Consequently we have not assessed the permeability of the grafted skin to lipophilic test penetrants.

In other studies (Reifenrath et al 1984), a good correlation has been reported between the permeability properties of human skin alone and grafted on athymic mice. In those studies human surgical specimens were available and grafted within 24 h of death. In our study, the tissue was not available until between 24 and 72 h after death. We have shown that such tissue, whilst appearing normal by histopathological assessment, shows abnormally enhanced permeability especially to cationic molecules. Our results indicate that such grafted tissue may not offer any advantage over existing techniques (Dugard et al 1984) which use human cadaver skin in-vitro for the prediction of human in-vivo absorption.

References

Blank, I. H. (1965) Cutaneous barriers. J. Invest. Dermatol. 45: 249–256

Davies, M. G., Vella Briffa, D., Greaves, M. W. (1979) Systemic toxicity from topically applied salicylic acid. Br. Med. J. 663

Dugard, P. H., Walker, M., Mawdsley, S. J., Scott, R. C. (1984) Absorption of glycol ethers through human skin in-vitro. Environ. Health Persp. 57: 193–197

Elias, P. M., Cooper, E. R., Korc, A., Brown, E. E. (1980) Importance of stratum corneum structural parameters Vs lipid composition for percutaneous absorption. J. Invest. Dermatol. 74: 247

Kearney, J. N., Gowland, G., Holland, K. T., Cunliffe, W. J. (1982) Maintenance of the normal flora of human skin grafts transplanted to mice. J. Gen. Microbiol. 128: 2431–2437

Kruger, G. G., Briggaman, R. A. (1982) The nude mouse in the biology and pathology of skin. In: Fogh, J., Giovanella, B. (eds) The Nude Mouse in Experimental and Clinical Research 2, Academic Press. pp 301–322

Reifenrath, W. G., Chellquist, E. M., Shipwash, E. A., Jederberg, W. W., Krueger, G. G. (1984) Percutaneous penetration in the hairless dog, weanling pig and grafted athymic mouse: evaluation of models for predicting skin penetration in man. Br. J. Dermatol III: 527, 123–135

Scheuplein, R. J., Blank, I. H. (1973) Mechanism of percutaneous absorption IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. J. Invest. Dermatol. 600: 286–296

Scott, R. C., Walker, M., Dugard, P. H. (1986a) A comparison of the in-vitro permeability properties of human and some laboratory animal skins. Int. J. Cosmet. Sci. 8: 189–194

Scott, R. C., Ramsey, J. D., Ward, R. J., Thompson, M. A., Noden, C., Rhodes, C. (1986b) Percutaneous absorption: in vitro assessment. Food. Chem. Toxicol. 24: No 6/7, 767–764

Scott, R. C., Walker, M., Dugard, P. H. (1986c) In-vitro percutaneous absorption experiments: a technique for the production of intact epidermal membranes from rat skin. J. Soc. Cosmet. Chem. 37: 35–41