The Influence of Different Strains and Age on *in Vitro* Rat Skin Permeability to Water and Mannitol

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Water and mannitol were used as test penetrants to study the effect of age on the skin permeability of the Wistar-derived Alderley Park (AP) rat and Sprague–Dawley (SD) rat. Whole-skin membranes were prepared from rats aged 10 to 120 days, while epidermal membranes were prepared from rats aged 24 to 32 days. The results indicated that the skin permeabilities of the two strains were very similar for either whole-skin or epidermal membranes. The influence of age on skin permeability was found to be negligible for the AP rat, and a small decrease in whole-skin permeability was observed for SD rats above 80 days of age. A statistically derived expression ("the separation efficiency factor") was used to determine the optimum age for preparing intact epidermal membranes; these were 26 days for AP rats and 28 days for SD rats. Histological examination of whole-skin membranes for both strains revealed that the stratum corneum and epidermal thickness did not alter significantly with age (10 to 120 days old). Dermal thickness, hair follicle depth, and, to a lesser extent, the surface area occupied by hair follicles all appeared to be influenced by age, although these changes had no detectable effect on skin permeability.

KEY WORDS: *in vitro;* percutaneous absorption; age; histology.

INTRODUCTION

Percutaneous absorption studies have helped to improve the therapeutic efficacy and identify the dermal toxicity of topical agents. With the ethical and practical problems associated with human experiments, a wide range of laboratory animal skin alternatives has been used to predict human skin absorption. As a result, the absorption rate for different chemicals has been compared using a common species (1,2) or several species (3,4). However, there has been little consideration of the effect that using different strains of the same species may have on comparisons of absorption data.

Structural changes in skin have been related to age (5–7), most notably between preterm and infant human skin (8,9). However, studies of the effect of age on skin permeability have produced conflicting evidence showing an increase (10–12), a decrease (13–19), or relatively no change (12,13,15,20,21) in permeability with an increase in age for human and animal skin membranes.

In this study, the *in vitro* absorption of two test penetrants, water and mannitol, was measured through whole-skin and epidermal membranes prepared from Wistar-derived Alderley Park (AP) rats and Sprague–Dawley (SD) rats. By measuring the permeability of rat skin over an extensive age range (10 to 120 days old), both interstrain comparisons and the assessment of the influence of age have been made. Whole-skin samples were also taken for histological examination of the structural development of rat skin for both strains over this age range.

MATERIALS AND METHODS

Chemicals

[^{14}C]Mannitol (sp act, 60 mCi mmol^{-1}) and [^{3}H]water (sp act, 270 mCi mmol^{-1}) were supplied by Amersham International, Amersham, UK. [^{14}C]Mannitol was diluted in distilled water to give a final activity of approximately 2.5 μCi ml^{-1} and made up to a concentration of 1 mg ml^{-1} with unlabeled mannitol (supplied by Sigma Chemical Co., Poole, UK). Tritiated water was diluted in 0.9% physiological saline to give a final activity of approximately 2.5 μCi ml^{-1}. Optiphase MP scintillation fluid was supplied by LKB and manufactured by FSA Laboratory Supplies (Loughborough, UK).

Membrane Preparation

Alderley Park rats (strain Alpk:APfSD) were supplied by the Barriered Animal Breeding Unit (Alderley Park, Cheshire, UK). Sprague–Dawley rats [strain Crl:CD (SD)BR] were supplied by Charles River UK Ltd. (Margate, Kent, UK). Whole-skin membranes were removed from the dorsal region and epidermal membranes were prepared using a chemical separation technique (22).

In Vitro Percutaneous Absorption Studies

Prepared membranes were mounted on horizontal-membrane static glass diffusion cells (exposure area, 2.54 cm^2) and maintained at 30°C in a water bath. A solution of 0.9% physiological saline was used as the receptor fluid. The integrity of the membranes was initially assessed by measuring the permeability of tritiated water (400 μl cm^{-2} skin, occluded, Day 1). The absorption of [^{14}C]mannitol was then studied (200 μl cm^{-2} skin, occluded, Days 2–4), and the steady-state absorption rates were calculated for each penetrant. From the absorption rate, a permeability coefficient (cm hr^{-1}) was calculated and this was the final expression of the permeability of each penetrant.

The "Separation Efficiency Factor"

This statistically derived formula was used to quantify the efficiency of preparing "intact" epidermal membranes from different aged rats. The separation efficiency factor (SEF value) was calculated at each age using the following expression:

$$\text{SEF} = \text{mean of } \frac{\text{No. of "intact" membranes per skin}}{\text{No. of membranes prepared per skin}} \times 100\%$$

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An epidermal membrane was deemed intact if it displayed a tritiated water permeability similar to that obtained using whole skin ($<2.5 \times 10^{-3}$ cm hr^{-1}) (22).

Histology

Histology was performed on whole-skin samples over the age range 10 to 120 days old. This included the examination of stratum corneum thickness, viable epidermal thickness, dermal thickness, hair follicle depth, and the skin surface area occupied by hair follicles.

RESULTS

Influence of Strain

Graphical comparisons of the permeability of AP rat and SD rat whole skins are presented in Fig. 1 for the two test penetrants over the age range 10 to 120 days old. The mean permeability coefficients (K_p) obtained for each strain of rat were compared at each age, treating each penetrant independently (two-sided Student's t test: $P < 0.05$ indicated by asterisks). The skin permeabilities of both strains of rat were very similar, and where significant differences were recorded, they were comparable to the magnitude of the variation in skin permeability found between rats of the same strain.

The overall mean K_p values (±SE) for whole-skin membranes calculated over the entire age range were also similar for both strains for water [AP rat = 1.43 (±0.05) × 10^{-3} cm hr^{-1}, n = 178; SD rat = 1.53 (±0.06) × 10^{-3} cm hr^{-1}, n = 150] and mannitol [AP rat = 3.23 (±0.17) × 10^{-4} cm hr^{-1}, n = 178; SD rat = 2.89 (±0.17) × 10^{-4} cm hr^{-1}, n = 150]. Interstrain similarities were also indicated by the overall mean K_p values obtained for epidermal membranes for water [AP rat = 1.16 (±0.08) × 10^{-3} cm hr^{-1}, n = 30; SD rat = 1.41 (±0.08) × 10^{-3} cm hr^{-1}, n = 22] and, to a lesser extent, mannitol [AP rat = 2.30 (±0.27) × 10^{-4} cm hr^{-1}, n = 30; SD rat = 0.89 (±0.15) × 10^{-4} cm hr^{-1}, n = 22].

Influence of Age

The mean K_p value at each age was statistically compared with the overall mean K_p value for the entire age range for each penetrant; any significant differences were highlighted by asterisks (two-sided Student's t test; $^*P < 0.05$, $^{**}P < 0.01$). Generally, age did not affect the skin permeability of either strain of rat for the test penetrants. For instances, where significant differences were recorded for AP rat skin permeabilities, the difference between the mean K_p at these ages and the overall mean K_p was less than a factor of two. However, for SD rats, there was an indication of a decrease in whole-skin permeability for 100- and 120-day-old animals, where the mean K_p's at these ages were significantly less than the overall mean K_p value for both penetrants.

The effect of age (24–32 days only) on the permeability of the test penetrants (mean K_p) through AP rat and SD rat epidermal membranes was also examined (Fig. 2). The mean K_p values did not differ significantly from the overall mean K_p value, although the permeability of water through 30-day-old AP rat epidermis was significantly higher ($P < 0.05$).

A "separation efficiency factor" (SEF value) for epidermal membrane preparation from AP rats and SD rats was calculated at each age. For AP rat epidermal membranes, the SEF value was greatest for 26-day-old rats, decreasing for older animals. A similar relationship between the SEF value and age was exhibited for SD rat epidermal membranes, the maximum SEF value occurring at 28 days of age.

Histology

Changes in the structure of skin as determined by histological examination were studied over the age 10–120 days. For both strains of rat, there was very little variation with age for stratum corneum thickness (range, 16.3–24.8 μm) and viable epidermal thickness (range, 14.0–20.1 μm). Age did influence the dermal thickness (range, 264 μm at 26 days for AP and 796 μm at 60 days for AP rats), minimum hair follicle depth (range, 144 μm for AP at 24 days and 734 μm

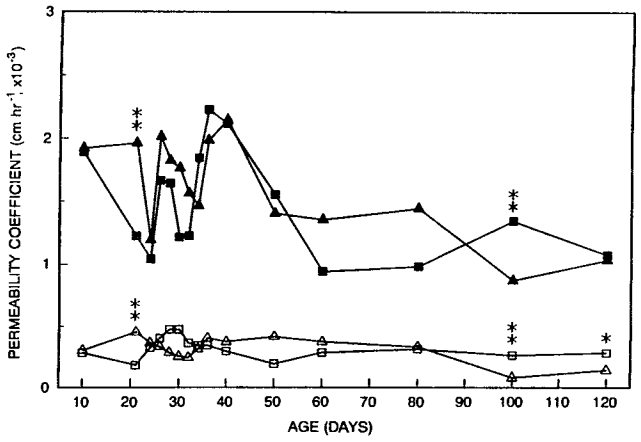

Fig. 1. The effect of age on the mean permeability coefficient (K_p) ± SE ($7 < n < 18$) for water and mannitol through AP rat whole skin. Two-sided Student's t test between the mean K_p at each age and the overall mean K_p: (*) $P < 0.05$; (**) $P < 0.01$. (■) AP/water; (▲) SD/water; (□) AP/mannitol; (△) SD/mannitol.

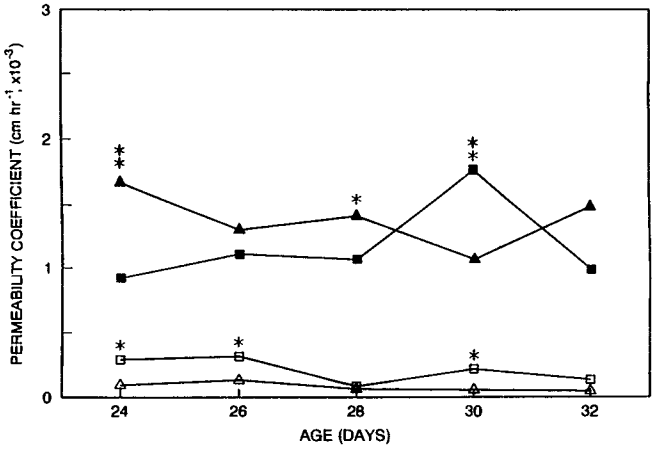

Fig. 2. An interstrain comparison of the mean permeability coefficient for water and mannitol through AP rat and SD rat epidermal membranes at each age. Two-sided Student's t test: (*) $P < 0.05$; (**) $P < 0.01$. (■) AP/water; (▲) SD/water; (□) AP/mannitol; (△) SD/mannitol.

at 36 days for SD rats), and the surface area occupied by hair follicles (range, 0.008 mm^2/mm^2 for SD rats at 120 days and 0.034 mm^2/mm^2 for SD rats at 10 days). Similar age-related changes in skin structure were exhibited by both strains of rat and this observation was supported by a statistical interstrain comparison of the histological data.

DISCUSSION

Wistar-derived Alderley Park rats and Sprague–Dawley rats are commonly used animals in this laboratory and are both outbred strains of albino rat. Ideally, for interexperimental comparisons, variables should be reduced to a minimum to enable valid and concise interpretation of the data. By comparing the skin permeability of two similar strains of rat, a basis could be established to assess the effect of using more varied rat strains. Using two test hydrophilic penetrants we have examined any differences between the skin permeabilities of the two rat strains over the age range 10–120 days.

It was of further interest to investigate interstrain permeability for the *in vitro* percutaneous absorption of lipophilic penetrants. Using the selected chemical separation technique, epidermal membranes could be physically prepared only from rats aged 24 to 32 days old, and then with varying degrees of success (indicated by the SEF values). Therefore, in order to investigate fully any change in *in vitro* skin permeability over the desired relatively wide age range, it was necessary to use whole-skin membranes and hydrophilic penetrants. It must be realized, however, that any apparent relationship might not be directly applicable to lipophilic molecules, and this will require further investigation. In addition, any patterns seen in an animal model such as the rat might not be reflected in humans.

A comparison of the permeability of whole skin and epidermis to water has previously been used as an indicator of the success of intact epidermal preparation (3,22). In this study, significant differences were reported between whole-skin and epidermal water permeabilities for AP rats aged 28 and 30 days and SD rats aged 24 days (two-sided Student's t test, $P < 0.05$). However, there was no significant difference between the water permeability for whole-skin and that for epidermal membranes at the suggested optimal ages for epidermal preparation (i.e., AP rat = 26 days and SD rat = 28 days; $P > 0.05$). Epidermal membranes are necessary to study the *in vitro* absorption of lipophilic penetrants, and the age of the animal concerned may be an important feature in the success of any chosen technique.

A number of reported studies have examined the effect of age on the skin permeability. Wester *et al.* (20) found similar amounts of testosterone absorbed through newborn and adult rhesus monkey skin. γ-Benzene hexachloride was absorbed through newborn guinea pigs faster than through 2-month-old animals, although the differences were not found to be statistically significant (14). Many studies have compared young and adult rat skin permeability (12,17–21). The studies used different dosing regimes (itself representing a factor that can affect rates of absorption), but there appears to be an overall indication that young rat skin is more permeable than adult rat skin; this evidence is, however, equivocal.

A study using male Fischer 344 rats demonstrated relatively consistent *in vitro* skin absorption of 2,3,4,7,8-pentachlorodibenzofuran over the age range 36 to 120 days and of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin over the age range 36 to 96 days (19). However, for both penetrants, where a constant dose per surface area was applied, there was a statistically significant increase in skin permeability for 10-day-old animals (up to a twofold difference). Workers using hairless mice found that, for both hydrocortisone (23) and a series of alkanols (24), *in vitro* skin permeability increased with age to a maximum at 25 days. This was followed by a rapid decrease in permeability over the next 25 days before an eventual leveling-off at about 100 days of age. This suggests that the permeability of hairless mouse skin is markedly more sensitive to the age of the animal than the permeability of the two rat strains used in this study.

From the data obtained in this study, age did not influence the permeability of whole-skin or epidermal membranes. For AP rat whole skin, the slope of the regression of the relationship between age and permeability to water and mannitol could not be distinguished from zero ($P > 0.05$), i.e., there was no linear relationship between skin permeability and age. For SD rat whole skin, the test for linear regression indicated that an inverse relationship may exist between age and skin permeability for both water and mannitol ($P < 0.01$), i.e., a decrease in permeability with increase in age. However, this relationship was lost if the data for 100- and 120-day-old rats were omitted. Rats older than this are not commonly used in percutaneous absorption studies, particularly *in vitro* studies, and consequently, they were not included in this series of experiments. The test for linear regression did not indicate any correlation between age and permeability for epidermal membranes for either strain ($P > 0.05$).

The stratum corneum is known to be the principal barrier in percutaneous absorption. The stratum corneum thickness of AP rats and SD rats was relatively unaffected by age, and this may have contributed to the consistency in the permeabilities of water and mannitol. Although changes in dermal thickness did occur with age, this would not be expected to affect the permeability of skin *in vivo*, as chemicals are usually absorbed into the vascular supply serving the epidermal–dermal junction.

For both the AP rat and the SD rat, changes in whole-skin thickness were attributed principally to changes in the dermal thickness, since the stratum corneum thickness and viable epidermal thickness were relatively constant. For AP rat and SD rat skin, a similar relationship was observed for dermal thickness and hair follicle depth over the age range 10 to 60 days. Although the dermal thickness increased slightly above 60 days of age, the corresponding hair follicle depth decreased.

Hair follicles have been considered as possible routes of absorption, particularly for slowly absorbed penetrants. Scott *et al.* (26) measured the hair follicle opening area obtained from different species and observed that the permeability of more slowly absorbed penetrants (mannitol and paraquat) increased as the follicle opening area increased, although faster-absorbed penetrants (water and ethanol) did not appear to be influenced by follicle opening area. In this study, the hair follicle opening area did seem to decrease up

to about 28 days of age for both AP rats and SD rats, although most values were between 0.010 and 0.025 mm^2 hair follicles mm^{-2} skin. These minor fluctuations in follicle area were not sufficient to influence the absorption of mannitol or water through the rat skin, although the decrease in follicle opening area for SD rats over 80 days old may be linked with the decrease in skin permeability already discussed.

The age band where the structural attributes of both AP rat and SD rat skin were most variable was between 10 and 36 days of age. This age band also corresponded to fluctuating mean K_p values for both strains of rat (Figs. 1 and 2). This suggests that changes in the structure of skin with age, in particular, hair follicle depth and dermal thickness, may have a minor influence on the percutaneous absorption of water and mannitol. However, the changes in skin permeability were too small to propose any positive relationship with skin structure.

CONCLUSIONS

The skin permeability properties of the two strains of rat (Alderley Park and Sprague–Dawley) were similar for water and mannitol over a wide age range for both whole-skin membranes (10 to 120 days old) and epidermal membranes (24 to 32 days old). Generally, there was no effect of age on the permeability to selected penetrants. However, the data indicated that the permeability of SD rat whole skin decreased above 80 days of age.

From the expression used to calculate the "separation efficiency factor," the optimum ages for preparing intact epidermal membranes from AP rats and SD rats (using a chemical separation technique) are 26 and 28 days, respectively.

The histology of the whole-skin samples revealed that both stratum corneum thickness and viable epidermal thickness remained relatively constant over the age range 10 to 120 days. However, dermal thickness, minimum hair follicle depth, and, to a lesser extent, the surface area occupied by hair follicles were all influenced by the age of the rat for both strains.

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