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Report

An Examination of Excised Skin Tissues Used for *In Vitro* Membrane Permeation Studies

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The morphology and layer thicknesses of excised human and hairless mouse skin have been examined. Excised tissues were prepared either by heating at 60°C or by incubation in an ethylenediaminetetracetic acid (EDTA) solution. Either process yielded spearated sheets of stratum corneum plus attached viable epidermis (SCE), the thicknesses of which were determined microscopically. These measurements indicated that initial skin separation occurred at the dermal/epidermal junction for both separation processes. The two techniques produced SCE sections showing consistent differences in thickness of the attached viable epidermis layer. This effect depended upon the presence or absence of epidermal invaginations. In the former case, EDTA-separated tissue gave narrower viable epidermis of more uniform thickness than that seen with heat-separated tissue. In the latter case, both techniques produced SCE having viable epidermis layers of similar thickness.

KEY WORDS: excised skin; preparation; dimensions; morphology.

INTRODUCTION

Excised skin tissues used in *in vitro* permeation studies can be prepared in a variety of ways. Most simply, the subcutaneous fat can be trimmed away from the subdermal surface to leave so-called "whole" skin. This comprises the topmost stratum corneum, the viable epidermis, and part of the dermis. The two underlying layers of this preparation may then be either partially or totally removed without substantially affecting permeation rates. In the former case, the dermis may be separated either by heat treatment at 60°C (1) or by incubation in an ethylenediaminetetracetic acid (EDTA) solution (2) to produce stratum corneum plus attached viable epidermis (SCE). Total removal of the dermis and the viable epidermis by digestion with trypsin leaves isolated stratum corneum (SC).

Conflicting evidence is available concerning the possible detrimental effects of heat separation upon the integrity of skin (3,4). Yet provided that the stratum corneum remains intact, heat-separated human SCE does function as a discriminating barrier to drug permeation (5). A stronger case can be made for the doubtful effects of trypsin treatment upon SC (6,7). These possible problems may be avoided quite easily by using SCE directly in permeation studies in place of SC. It is then assumed that the viable epidermis layer of this tissue has little influence upon measured drug permeation rates (5).

There is some uncertainty about the exact morphology of the SCE. Although its separation from the dermis is assumed to occur at the epidermal/dermal junction, this has not been confirmed by measurements of the thicknesses of the tissue layers present. Such measurements available in the literature deal only with whole skin (8); we could not find values for the layer thicknesses existing within SCE. Bearing in mind the application of either heat or EDTA during its preparation, the possibility for morphological change and variation in layer thickness of the SCE is clear. Consequently, we felt it advisable to undertake a microscopic examination of excised SCE prepared from both humans and hairless mice. The SCE was prepared by the two techniques mentioned above, namely, heat treatment and EDTA incubation.

MATERIALS AND METHODS

Preparation of Excised Human SCE

Samples of whole Caucausian human skin were excised from the midline chest of cadavers within 48 hr postmortem. Skin from five different cadavers was used, the donors having died at ages from 51 to 94 years. Any subcutaneous fat was carefully trimmed from the subdermal surface. Half of the sample was then immersed in water for 2 min at 60 ± 1°C. The remaining half was incubated for 3.5–5.5 hr at 37 ± 1°C in a 0.76% (w/v) aqueous solution of EDTA (Fisher Scientific, Fair Lawn, N.J., Lot 853480) in phosphate-buffered saline. After either treatment the SCE could be peeled away from the underlying dermis as a single sheet of tissue.

Preparation of Excised Hairless Mouse SCE

Samples of hairless mouse skin (female, 16–18 weeks

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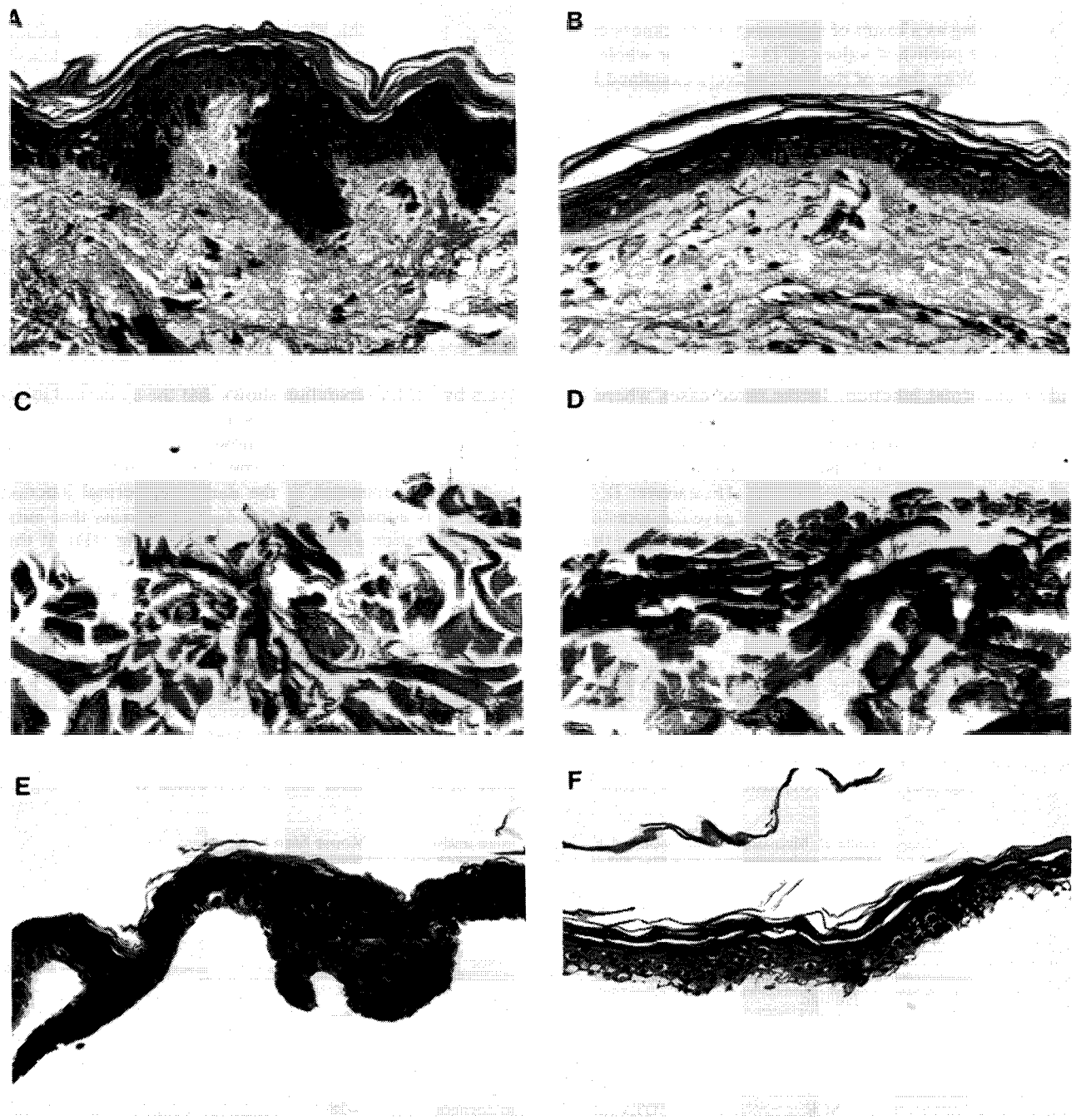

Fig. 1. Transverse sections through excised human skin. (A) Whole skin before separation (cadaver I); (B) whole skin before separation (cadaver III); (C) dermal remains after heat separation (cadaver I); (D) dermal remains after EDTA separation (cadaver I); (E) SCE after heat separation (cadaver I); (F) SCE after EDTA separation (cadaver I).

old, Temple University, Philadelphia, Pa.) were excised from the backs of four mice immediately after their death by cervical dislocation. In this case SCE could be prepared only by EDTA immersion for 3 hr, as described above. Intact sheets of SCE could not be separated by heating but remained firmly attached to the dermis.

Microscopic Examination

Tissue samples were left soaking for a minimum of 24 hr in formalin, after which they were embedded in molten wax. After cooling, transverse sections 6–7 μm in thickness were cut with a microtome. The wax was removed with ethanol and xylene, and each section treated with hematoxylin and eosin stain before being mounted and examined under 400× magnification. The thicknesses of the stratum corneum and viable epidermis layers were determined using a calibrated objective graticule.

RESULTS

The whole human skin sections (Fig. 1A) show quite clearly their layered structure, comprising the stratum corneum, the darkly stained viable epidermis, and the lightly

stained dermis. Although the stratum corneum shows the usual shredding as a result of sectioning, its thickness corresponds to the published values of 15–16 μm for whole skin (Table I) (8). With three of the five cadavers examined here, the typical invaginations of the viable epidermis into the underlying dermis are seen (typified by cadaver I; Fig. 1A). The remaining two cadavers showed no such invaginations (typified by cadaver III; Fig. 1B), a characteristic of aged skin. Examination of the ages of the donors at their times of death does not, however, reveal an obvious correlation between age and the absence of invaginations (footnotes to Table I).

After treatment either by heat or with EDTA, no darkly stained epidermal tissue can be seen remaining attached to the dermal remains (Figs. 1C and D, cadaver I). The whole skin tissues thus appear to have separated at, or close to, the epidermal/dermal junction. In the three cases where epidermal invaginations were seen in the whole skin sections, they were also observed as part of the SCE after heat separation (Fig. 1E, cadaver I). However, they were not seen as part of the EDTA-separated SCE (Fig. 1F, cadaver I). As a result, the viable epidermis of the latter gave the appearance of being thinner and of a more uniform thickness than that of the heat-separated SCE. For the two cases where no epidermal invaginations were present in the whole skin sections, the viable epidermal layers of the heat- and EDTA-separated SCE sections were of similar thickness. These observations are reflected in the measured ranges of thickness of the viable epidermis layers in these various sections (Table I).

No invaginations of the epidermis were seen with either the hairless mouse whole skin (Fig. 2A) or the EDTA-separated SCE (Fig. 2C) sections. Separation of the whole skin by EDTA evidently occurred at the dermal/epidermal junction (cf. Fig. 2A with Fig. 2C). The resulting SCE sections (Fig. 2C) show layers of viable epidermis very close in thickness to those of the original whole skin (Table I).

DISCUSSION

In the cases where epidermal invaginations are seen in the human whole skin sections, they are still observable in the viable epidermis layer of the SCE separated by heat treatment (Figs. 1A and E). It is evident from the dermal remains that heat separation occurred at the dermal/epidermal junction (Fig. 1C). These findings are in agreement with Kligman and Christophers' original assumption that the viable epidermis is separated "intact" by the heating process (1). However, the SCE prepared from the same cadavers by EDTA separation shows that the epidermal invaginations of the viable epidermis are no longer present (Fig. 1F). The action of EDTA is known to interupt the cation-mediated binding of the epidermis to the dermis (9), which should allow separation at the dermal/epidermal junction. Indeed, it is apparent from the dermal remains that initial EDTA separation did occur at this level (Fig. 1D). It thus appears that the EDTA treatment caused some part of the tissue associated with the epidermal invaginations to become detached from the SCE after initial separation. One would expect that this effect should not occur with the cadavers whose whole skin originally showed no epidermal invaginations (Fig. 1B for cadaver III). Indeed, both the heat- and the EDTA-separated SCE prepared from these cadavers shows viable epidermal layers of comparable thickness to that of their original whole skin sections. There is no evidence of a substantial loss of epidermal tissue during the

Table I. Measured Dimensions of Excised Human and Hairless Mouse Skin Sections

Source and section	Separation treatment	Tissue	Range of thickness (μm) Cadavers I, II, V[a]	Cadavers III, VI[b]
Human				
Whole skin	—	Stratum corneum	8–28	11–20
		Viable epidermis	19–130	15–58
SCE	Heat	Stratum corneum	6–18	9–13
		Viable epidermis	17–105	9–58
SCE	EDTA	Stratum corneum	5–18	11–20
		Viable epidermis	10–30	5–58
Dermis	Heat	Viable epidermis	0	0
Dermis	EDTA	Viable epidermis	0	0
			Four mice	
Mouse				
Whole skin	—	Stratum corneum	5–8	
		Viable epidermis	9–23	
SCE	EDTA	Stratum corneum	4–10	
		Viable epidermis	8–20	
Dermis	EDTA	Viable epidermis	0	

[a] Cadavers showing epidermal invaginations. Ages upon death: I, 94 years; II, 72 years; V, 59 years.

[b] Cadavers showing no epidermal invaginations. Ages upon death: III, 51 years; VI, 74 years.

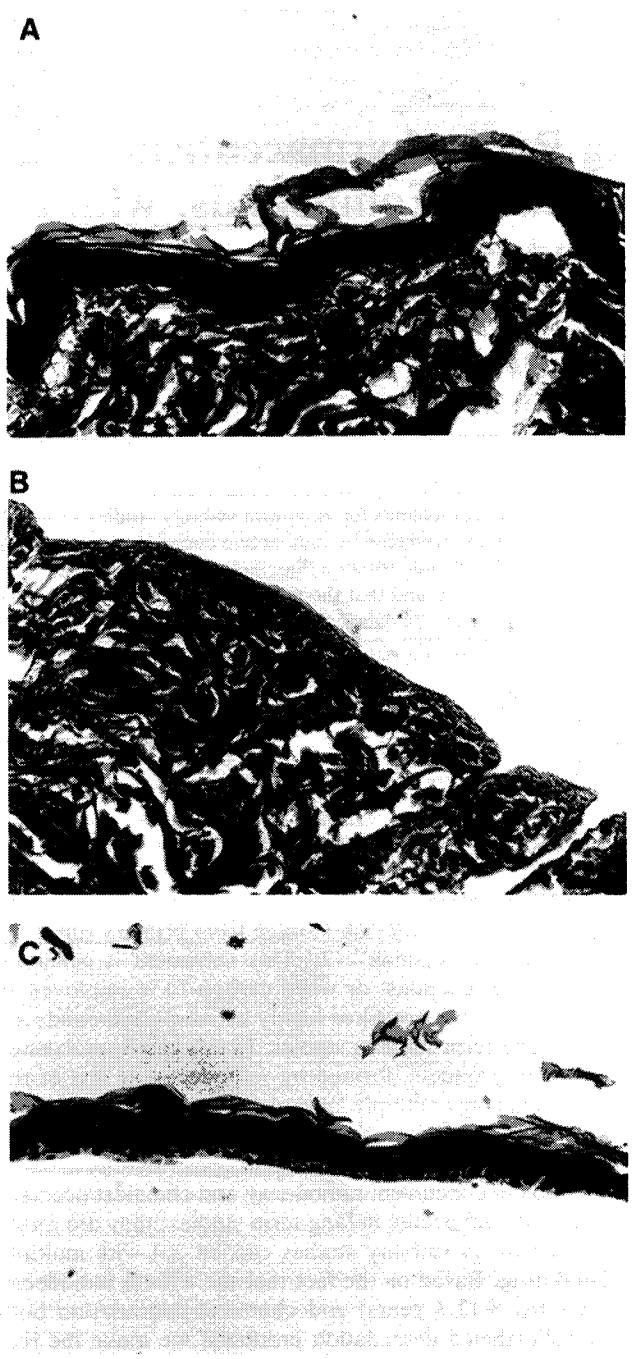

Fig. 2. Transverse sections through excised hairless mouse skin. (A) Whole skin before separation; (B) dermal remains after EDTA separation; (C) SCE after EDTA separation.

EDTA treatment in these cases (Table I). Similarly, since the hairless mouse whole skin sections show no invaginations (Fig. 2A), the epidermal layer of the resulting EDTA-separated SCE is of equivalent thickness to that of the whole skin (Table I).

Since handling of skin during any separation process will likely vary from person to person, care should be exercised in drawing general conclusions from a single study such as this. A consistent pattern of behavior does emerge, however, from the results presented here. Differences between the SCE sections prepared by the two techniques are apparent, apparently depending on whether or not the original whole skin showed epidermal invaginations. As a result of the apparent loss of some epidermal tissue during EDTA separation, the resulting SCE shows a viable epidermis of a thinner and more uniform thickness than that seen with heat-separated SCE.

As a general observation, both the EDTA- and the heat-separated SCE appears structurally intact. A comparison of the permeabilities of drugs through SCE prepared by the two techniques will be presented subsequently.

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REFERENCES

1. A. Kligman and E. Christophers. *Arch. Dermatol.* 88:702–705 (1963).
2. K. Baker and M. Habowsky. *J. Invest. Dermatol.* 80:104–107 (1983).
3. C. Behl, G. Flynn, T. Kurihara, W. Smith, O. Gatmaitan, W. Higuchi, N. Ho, and C. Pierson. *J. Invest. Dermatol.* 75:340 (1980).
4. M. Lehman. U.S. Army Chemical Research and Development Laboratories Special Publication 2–56, p. 10 (1964).
5. J. Swarbrick, G. Lee, and J. Brom. *J. Invest. Dermatol.* 78:63–66 (1982).
6. S. Rothbert and G. Axilrod. *Biochem. Med.* 2:1 (1981).
7. H. Jensen and N. Mottet. *J. Cell Sci.* 6:511 (1970).
8. R. Bronaugh, R. Stewart, and E. Congdon. *Toxicol. Appl. Pharmacol.* 62:481–488 (1982).
9. L. Scaletta and D. MacCallum. *Am. J. Anat.* 133:431–439 (1972).