Human Cadaver Skin Viability for *In Vitro* Percutaneous Absorption: Storage and Detrimental Effects of Heat-Separation and Freezing

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Received June 30, 1997; accepted October 7, 1997

Purpose. For decades, human cadaver skin has been banked and utilized by hospitals for burn wounds and to study percutaneous absorption and transdermal delivery. Skin storage maintenance and confirmation of skin viability is important for both uses, especially for the absorption process where the *in vivo* situation is simulated.

Methods. Our system uses dermatomed human cadaver skin immediately placed in Eagles MEM-BSS, and refrigerated after donor death, then transfered to the laboratory and placed in Eagles MEM-BSS with 50 μg/ml gentamicin at 4°C for storage.

Results. Skin viability, determined by anaerobic metabolism where glucose is converted to lactose, was highest ($p<0.000$) during the 18 hours of the first day after donor death, decreased some 3-fold by day 2 ($p<0.000$), but then maintained steady-state viability through day 8. Viability then decreased by approximately one-half by day 13. Thus, using the above criteria, human skin will sustain viability for 8 days following donor death in this system. Heat-treated (60°C water for one minute) and heat-separated epidermis and dermis lose viability.

Conclusions. Human skin viability can be maintained for absorption studies. It is recommended that this system be used, and that heat-separation and skin freezing not be used, in absorption studies where skin viability and metabolism might be contributing factors to the study.

KEY WORDS: human skin; viability; storage; freezing; heat-separation.

INTRODUCTION

Human cadaver skin is utilized in hospitals and research laboratories for various reasons. For nearly four decades, hospitals have banked skin for use as an effective temporary covering for burn wounds (1). Research laboratories also use cadaver skin to study the percutaneous absorption of drugs (2) and hazardous chemicals of environmental concern (3). Procedures such as heat treatment to separate epidermis from dermis are performed as part of the skin preparation for these studies. Human cadaver skin is not an easily obtained commodity, and storage for use becomes a necessity. Refrigeration of, and freezing skin are commonly done. With treatment and storage, skin viability has become a concern. This study determined human cadaver skin viability from point of death through time of storage, and the effect of heat and freezing treatment.

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MATERIALS AND METHODS

Human cadaver skin was obtained from the Northern California Transplant Bank (San Rafael, CA). All donors were caucasian, aged 21–53 years, and both genders were represented. The time of a subject's death was recorded; skin was taken from the subject's thighs by use of a dermatome targeted to 500 micrometers. The skin was immediately placed in Eagles Minimum Essential Media (MEM) with Earle's Balanced Salt Solution (BSS) (*In Vitro* Scientific Products Corp., St. Louis, Missouri) and refrigerated at 4°C. The skin was then transported on ice to the laboratory and stored refrigerated at 4°C in Eagles MEM-BSS with 50 μg/ml gentamicin until used.

Dermatomed skin samples were mounted in an *in vitro* assembly consisting of flow-through design glass diffusion cells (Laboratory Glass Apparatus, Inc., Berkeley, CA). Eagles MEM-BSS with gentamicin served as receptor fluid and flow rate was 1.5 ml/hr. The receptor fluid was at 37°C; skin surface temperature at 32°C. Eagles MEM contains glucose, and glucose metabolism to lactate in anaerobic energy metabolism was used as the measure of skin viability. Lactate production was determined using the Sigma Diagnostic Kit No. 826-UV (St. Louis, MO) and a Hitachi spectrophotometer (San Jose, CA).

Dermatomed skin was used as stored in the refrigerator, frozen at −22°C for storage, or heat separated (60°C water for 1 minute) into epidermis and dermis.

RESULTS

Figure 1 shows lactate production from four human skin sources mounted in the diffusion system. Each data point represents four hour receptor fluid collection intervals over the 24 hour diffusion period. No chemical was dosed on the skin; just receptor fluid perfusing the skin. Lactate was produced by the skin sources over the full 24 hour period. The lactate curves rise in the early part of the period, where glucose is diffusing into the skin and lactate is diffusing out of the skin. Two skin

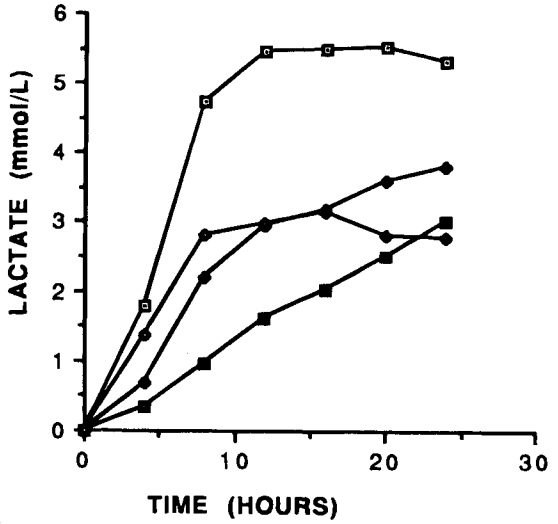

Fig. 1. Lactate production from glucose (anaerobic energy metabolism) of four human skin sources perfused for 24 hours with Eagles MEM-BSS with 50 μg/ml gentamicin. An initial time delay is noted where glucose absorbs into skin and lactose perfuses out of skin.

0724-8741/98/0100-0082$15.00/0

Table 1. *In Vitro* Human Skin Viability: Glucose Energy Metabolism to Lactate

Time after death (days)[a]	Dermatomed Skin		Heat-Separated Epidermis	
	Lactate[b] (m mol/L/24hr)	Number[c]	Lactate[b] (m mol/L/24hr)	Number[c]
0.75	19.8 ± 8.9[d,e]	6/2	2.0 ± 1.1[a,f]	3/1
2	5.9 ± 4.1[d]	13/4	1.8 ± 0.8[f]	8/3
3	8.0 ± 4.8	8/3	0.6 ± 0.5	6/2
4	6.5 ± 1.7	9/3	0.7 ± 0.4	6/2
6	6.8 ± 3.0	11/3	0.2 ± 0.1	5/2
8	4.6 ± 2.3	6/2	0.2 ± 0.1	3/1
13	2.0 ± 0.6	3/1	0.9 ± 0.4	3/1

[a] Stored refrigerated in Eagles MEM-BSS with 50 μm/ml gentamicin.
[b] Mean ± SD.
[c] Number of skin samples/number of human skin donors.
[d] $p<0.000$.
[e] $p<0.01$.
[f] $p<0.007$.

sources reached steady-state at about 12 hours; lactate from the other two skin sources continued to rise until the process was stopped at 24 hours.

Table 1 and figure 2 give the cumulative lactate produced (m mol/L) for the 24 hour perfusion period. Human skin was either dermatomed skin or heat-separated epidermis used within the time period of 0.75 to 13 days after donor death. The number of skin samples from the number of skin donors for each time period are also listed. With dermatomed skin, refrigerated for only 0.75 days, the 24 hour cumulative lactate was a high of 19.8 ± 8.0 m mol/L. Lactate production decreased by day 2 ($p<0.000$) and remained steady through day 8. Lactate production decreased further by approximately one half between day 8 and day 13. Heat-separated epidermis lactate production was less than dermatomed skin ($p<0.01$) at 2.0±1.1 m mol/L. This level was maintained to the 2 day period, then decreased

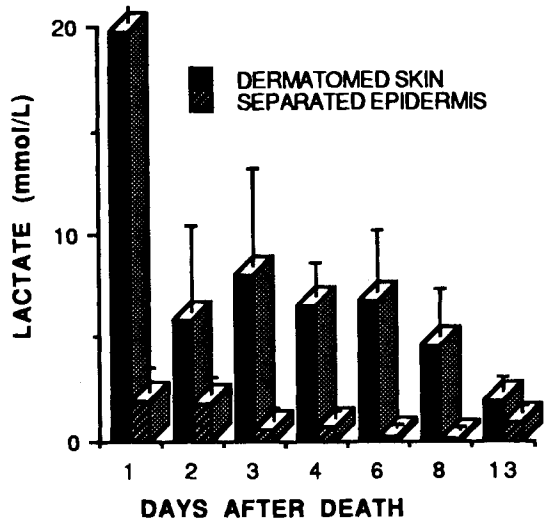

Fig. 2. Viability of human skin stored refrigerated at 4°C in Eagles MEM-BSS with 50 μg/ml gentamicin. Time indicated is that after donor's death.

Table 2. Heat Effect on Human Skin Simulating Epidermis Heat-Separation Procedure

Source	Lactate (m mol/L) Produced in 24 hours		
	Control Dermatomed Skin	Heat Treated Skin[c]	Statistics
#1[a]	13.6 ± 1.5	1.5 ± 0.6	$p<0.000$
#2[b]	7.4 ± 4.0	0.7 ± 0.14	$p<0.04$

[a] Male, 21, thigh skin, used 69 hours after death (n=3).
[b] Female, 30, thigh skin, used 39 hours after death (n=3).
[c] Heated for 1 minute in 60°C water.

($p<0.007$) at day 3 and remained less than 1 m mol/L through the 13 day test period.

Dermatomed skin was heat-treated at 60°C for one minute to simulate the heat-separation procedure to produce epidermis separated from dermis (but no separation was performed) (Table 2). Lactate production decreased significantly ($p<0.000$; $p<0.04$) for both heat-treated skin samples. Therefore, heating to separate epidermis from dermis damages viability. In another study (Table 3) lactate production was determined in heat-separated epidermis and dermis. The cumulative lactate production was much less than intact dermatomed skin, again showing the detrimental effect of heat-separation on skin viability.

Table 4 shows replicates from 6 dermatomed skin and heat-separated epidermis samples that were frozen at −22°C. The process of freezing was detrimental to skin viability of dermatomed skin ($p<0.04$). Separated epidermis was not significantly different between refrigerated and frozen ($p>0.05$)

Table 3. Viability of Dermatomed Human Skin, Heat Separated Epidermis and Dermis

Time (hours)	Lactate (m mol/L/24 hours) Production		
	Dermatomed Skin[a]	Epidermis[a]	Dermis[a]
29	9.7 ± 2.3[b]	1.5 ± 0.4[b]	0.9 ± 0.4[b]

Note: Epidermis plus dermis does not equal intact skin.
[a] Mean ± SD; male age 25, thigh skin.
[b] $p<0.004$.

Table 4. Freezing Effect of Human Skin on Energy Metabolism

Skin Sample	Lactate Production (m mol/L/24 hours)		Heat-Separated Epidermis	
	Dermatomed Skin			
	Refrigerated[b]	Frozen[a,b]	Refrigerated	Frozen[a]
1	12.2 ± 2.1	0.1 ± 0.1	1.0 ± 0.08	0.19 ± 0.13
2	2.4 ± 0.7	0.4 ± 0.3	1.5 ± 0.6	0.18 ± 0.13
3	7.4 ± 4.0	2.6 ± 0.4	—	—
4	9.7 ± 2.3	2.4 ± 0.5	1.5 ± 0.4	2.1 ± 0.2
5	9.5 ± 0.4	1.2 ± 0.04	0.2 ± 0.1	0.1 ± 0.05
6	27.5 ± 4.1	0.0	0.2 ± 0.1	0.0

[a] Frozen 24 hours or longer.
[b] $p<0.04$.

because the heat-separation process to get the epidermal layer had already been detrimental to skin viability.

DISCUSSION

It is logical that prolonged life and improved quality for stored skin is desirable for any transplant situation (4). During *in vivo* percutaneous absorption and transdermal delivery, the skin is viable and does metabolize glucose for energy, and metabolism does extend to other enzymes and other chemicals (5,6). Understanding and maintaining human cadaver skin viability places the skin use closer to the *in vivo* situation. This study shows that, in a sustaining media, skin can be energy viable for up to 8 days. Harvesting the skin and use within a day of donor death gives the highest viability. Our system gets the skin quickly into sustaining media and refrigeration; it is not known if a delay from harvest to storage will affect viability. Common practices of freezing skin for storage, or heat-treatment to separate epidermis from dermis, can destroy skin viability. The effect of enzymatically separating skin is not known. Bhatt et al (7) also showed that heat treatment of hairless mouse skin for separation purposes eliminates viability.

Cadaver skin viability can be maintained and monitored. Glucose utilization can be measured by conversion of [^{14}C]-glucose to $^{14}CO_2$ (8) or by lactate production (9) as shown here. The lactate production methodology does not require radioactivity use equipment.

ACKNOWLEDGMENTS

We thank the Northern California Transplant Bank who supplied the skin and made this study possible. Special acknowledgement to George Kositzin for the extra effort to obtain the skin samples.

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