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Effects of freezing on human skin permeability

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The percutaneous absorption of water was measured in-vitro at 30 °C for pale caucasian abdominal skin which had been stored at −20 °C for up to 466 days and compared with fresh skin. Prolonged freezing of the skin did not affect the absorption of water which had a mean permeability coefficient of $1{\cdot}71 \pm 0{\cdot}62 \times 10^{-3}$ cm h^{-1} (180 diffusion experiments with 39 skin specimens). No significant difference was found between the absorption of water through human skin which was fresh or had been frozen. The mean permeability coefficient for skin which had not been frozen was $1{\cdot}30 \pm 0{\cdot}55 \times 10^{-3}$ cm h^{-1} for 6 skin specimens.

In developing topical drug formulations and assessing toxic hazards, we often need to do in-vitro diffusion experiments through skin. Supplies of human skin are frequently small and erratic, and therefore it may be necessary to store suitable skin until it is required. Such storage must not affect the permeability of the skin if diffusion results are to correlate with in-vivo permeability. The most frequently used method of storage is freezing (Allenby et al 1969; Cohen & Stoughton 1974; Pitman & Rostas 1982). Burch & Winsor (1944) showed that there was no apparent influence of freezing and thawing on the permeability of skin to water for up to 3 weeks after death. Similarly, Astley & Levine (1976) reported that up to 6 months storage at −20 °C leaves human skin permeability unaffected. However, Swarbrick et al (1982) found for three skin specimens that freezing increased the absorption of a chromone acid compared with fresh or dried human skin.

We have examined whether prolonged freezing affected human skin permeability to water, a model polar penetrant, and compared the permeability of frozen skin with unfrozen samples used immediately after post mortem.

Materials and methods

Pale, caucasian, male and female skin (mean age 71 ± 11 years) taken from the abdomen at autopsy was sealed in evacuated plastic bags and frozen at −20 °C. Before the diffusion experiments the skin was flattened in a press and the epidermis and upper layers of the dermis were removed using a dermatome (Davies Duplex Electro Dermatome 7). Fresh skin was stored at 10 °C and it was used within 2–3 days of autopsy. The dermatomed skin, thickness ≈0·4 mm, was hydrated for 24 h before tritiated water diffusion was measured using standard glass diffusion cells (Scheuplein 1965). The tritiated water (the Radiochemical Centre, Amersham) was diluted to ≈4 μCi ml^{-1} before being added to the donor compartment of the diffusion cell. Samples (70 μl) were removed from the receptor and replaced by fresh 0·9% NaCl (saline) at hourly intervals between 6 and 9 h from the start of the experiment. The diffusion cells were maintained at 30 °C and up to 6 replicates were measured for each specimen at one time.

Results and discussion

Water diffusion results are in Fig. 1. The permeability coefficient (Kp) was calculated using the equation:

$$Kp = J/\Delta C$$

where J is the steady state flux and ΔC is the concentration gradient across the stratum corneum. The mean Kp ± one standard deviation is plotted for specimens measured at times within each 50 day period of freezing up to 466 days. The numbers in parentheses are the number of specimens tested and the total number of replicates measured in each time interval. The number of diffusion experiments with frozen skin was 180 and 39 specimens were used. The small variations in mean Kp values for frozen skin due to inter-specimen variation and experimental error are

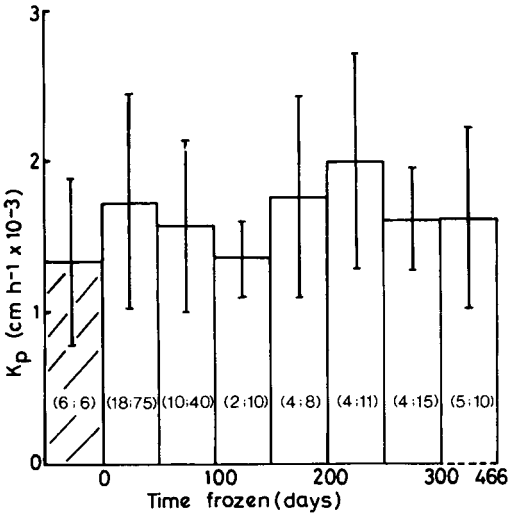

FIG. 1. Effect of prolonged freezing on human skin permeability to water. Mean permeability coefficients (Kp) ± one standard deviation are given for 50 day intervals. Numbers in parentheses are (number of skin specimens; total number of replicates). The hatched bar represents results for skin that was not frozen.

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well within one standard deviation of the overall mean Kp of $1{\cdot}71 \pm 0{\cdot}62 \times 10^{-3}$ cm h^{-1}. The mean Kp ($1{\cdot}33 \pm 0{\cdot}55 \times 10^{-3}$ cm h^{-1}) for water permeability through 6 specimens of skin which were not frozen before use is represented in Fig. 1 by the hatched bar. Student's *t*-test showed no significant difference between human skin permeability to water whether the skin had been frozen or not. The water Kp's obtained compare well with published values: Scheuplein (1971) quoted water permeability measurements through abdominal skin at 30 °C which give a Kp of $1{\cdot}2 \times 10^{-3}$ cm h^{-1} (when corrected for skin area), and the mean Kp for water permeability through human leg skin at ≈32 °C is $1{\cdot}3 \times 10^{-3}$ cm h^{-1} from work by Astley & Levine (1976).

In conclusion, the permeability of human post mortem skin to water (and probably other compounds for which water is a suitable model) is largely unaffected by freezing and thawing, even after prolonged freezing for up to 466 days. Also there is no trend to suggest that it will alter quickly at even longer times.

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Acetylcholine-induced inhibition of responses to field stimulation in rabbit pulmonary artery is unaffected by endothelium removal

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Acetylcholine (0·01–10 μmol $litre^{-1}$) relaxed normal rings (endothelium-retained) of rabbit pulmonary artery precontracted with clonidine (10 μmol $litre^{-1}$) while preparations with the endothelium removed responded with contraction only. Removal of the endothelium had no effect on contractions of the preparation to clonidine or field stimulation of the adventitial nerves (2 Hz, 10 s). Furthermore, the inhibitory effect of acetylcholine (0·3 and 1·0 μmol $litre^{-1}$) on contractions induced by field stimulation was not influenced by the vascular endothelium.

The ability of acetylcholine to diminish contractions induced by α-adrenoceptor agonists and several other drugs in isolated vascular preparations has recently been shown to be dependent on the presence of vascular endothelium (Furchgott & Zawadzki 1980; Chand & Altura 1981; De Mey & Vanhoutte 1981). It has been proposed that acetylcholine liberates a substance from the endothelium which in turn acts on the smooth muscle to mediate relaxation (Furchgott et al 1981). The release of this endothelial substance by acetylcholine is dependent on muscarinic cholinoceptor activation since the relaxant effect of acetylcholine is antagonized by atropine (Furchgott et al 1981). In vascular preparations in which the endothelium has been removed, acetylcholine produces only contraction, presumably by acting directly at muscarinic cholinoceptors on the smooth muscle cells (Vanhoutte 1974).

In addition to its direct and indirect effects on vascular smooth muscle, acetylcholine has been shown to inhibit sympathetic transmission in several blood vessels (Malik & Ling 1969; Rand & Varma 1970; Allen et al 1972). It is clear that this effect of acetylcholine is due to inhibition of transmitter release from the terminal noradrenergic varicosities and that the effect involves the activation of muscarinic receptors associated with the varicosities (Allen et al 1972; Steinsland et al 1973; Vanhoutte et al 1973). However, in view of the finding that the relaxant effect of acetylcholine on the vascular smooth muscle is mediated by a substance released from endothelial cells, it is possible that the inhibitory effect of acetylcholine on noradrenergic transmission might also be mediated or influenced by this substance. The present experiment was designed to investigate this possibility.

Methods and materials

Rabbits of either sex, 2 to 4 kg were killed by cervical dislocation and the main pulmonary artery was excised and trimmed of adhering connective tissue. Throughout, care was taken to avoid unintentional rubbing of the intimal surface of the preparation against foreign surfaces or itself. Rings of 0·5 cm length were cut from the artery and mounted vertically on two stainless steel hooks in a 15 ml organ bath, the upper hook being connected to either a tension or a displacement transducer. The bathing fluid was Krebs-Henseleit solution of the following composition (mmol $litre^{-1}$): NaCl, 119; KCl, 4·7; $NaHCO_3$, 2·5; $MgSO_4$, 0·45; KH_2PO_4, 1·0; $CaCl_2$, 2·5; D(+)-glucose, 11·1; EDTA, 0·067; ascorbic

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