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Permeation of linoleic acid through skin in vitro

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The syndrome of essential fatty-acid (EFA) deficiency is being recognized in persons suffering from fat malabsorption and in patients maintained on fat-free parenteral nutrition. Thus, Press et al (1974) reported that repeated cutaneous application of EFA rich oil reverses the plasma biochemical manifestation of EFA deficiency in man. However, Hunt et al (1978) failed to reverse deficiency in neonates by topical application of even higher doses of EFA-rich oil. Although EFA deficiency can be reversed or prevented using intravenous fat emulsions, the efficiency of topical essential fatty-acids is a relevant question because of the risks and inconveniences of infusion therapy.

A scaly condition of the skin and abnormally high rate of transepidermal water loss are characteristic symptoms of EFA-deficiency. Recently, Elias et al (1980) and Houtsmuller & Beek (1981) found that topical application of free linoleic acid is capable of improving the barrier function locally on EFA-deficient animals without prior correction of the systemic deficiency state. The mode of action was suggested to be by a specific effect of linoleic acid rather than a secondary response to normalized prostaglandin levels.

Based on the stated effect of topical linoleic acid on EFA deficiency abnormalities, the present experiments were undertaken to determine the permeability of rat skin and human skin to free linoleic acid.

Method

Whole abdominal human skin and skin from hairless rats, removed of all subcutaneous fat, were mounted in open diffusion cells. The epidermal side of the skin, having an available diffusion area of 1·8 cm^2, was exposed to ambient laboratory conditions. The dermal side was bathed by the receptor medium being stirred and kept at 37 °C. The receptor consisted of 7·5 ml 0·05 M phosphate buffer at pH 7·4 with 0·5% Pluronic F68 and 0·01% buthylhydroxytoluene (BHT). Pluronic and BHT were added to increase lipid solubility of the receptor phase and prevent linoleic acid oxidation, respectively. 100 ml of an ethanol solution containing 340 μg of 1-^{14}C-labelled linoleic acid and 0·01% BHT was spread across the epidermal surface where the ethanol was allowed to evaporate. At appropriate times samples were withdrawn and replaced by fresh receptor medium keeping an infinite sink. The amount of linoleic acid penetrating the skin was determined by measuring the radioactivity by a liquid scintillation counter. At the end of each experiment the skin was rinsed with chloroform, and the linoleic acid content within the skin was determined. The total skin uptake was considered as the sum of the linoleic acid content within the skin and the cumulative amount of linoleic acid in the receptor medium in 95 h.

Results and discussion

Fig. 1 represents a plot of data for the permeation of linoleic acid across excised human skin and hairless rat skin. After 4–5 h the data followed an almost linear relationship. The slope of the lines representing a pseudo-steady state flux of linoleic acid obtained for human skin and rat skin was 0·036 μg cm^{-2} h^{-1} and 0·098 μg cm^{-2} h^{-1}, respectively. The rat skin was found to be about three times more permeable to linoleic acid than abdominal human skin.

The rat skin data in Fig. 1 are derived from skin isolated from four different areas of the same animal, from the sides, the abdominal and the back area. The permeation rate through the various sections is shown in Fig. 2. Although the results are based on a single animal, it is noticeable that the permeation rate through the section from the underside of the animal was about double the rate of that through the back region. Such regional variation in percutaneous permeation is of practical importance in drug screening studies of topical absorption. This is in accordance with the observations of Horhota & Fung (1978) who found significantly

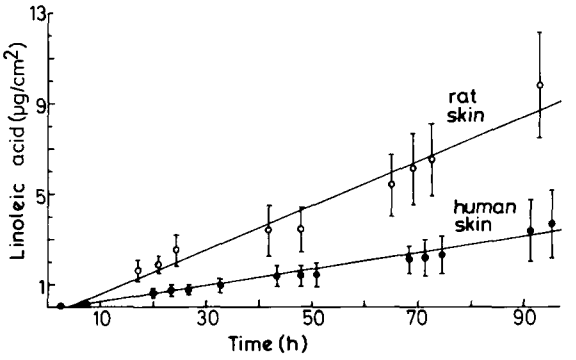

FIG. 1. Permeation of linoleic acid across nonhydrated excised human skin and hairless rat skin. Each point represents a mean value ± s.d. of four determinations.

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Table 1. Percutaneous permeation of linoleic acid in vitro. Applied dose of 185 μg cm^{-2}.

		In 95 h			
	Flux μg cm^{-2} h^{-1}	Total permeation μg cm^{-2}	Amount in skin μg cm^{-2}	Total skin uptake μg cm^{-2}	% dose
Human skin	0·036 ± 0·014	3·7 ± 1·5	12·1 ± 3·1	15·8	8·6
Hairless rat skin	0·098 ± 0·027	9·8 ± 2·3	96·0 ± 22·2	105·8	55·6

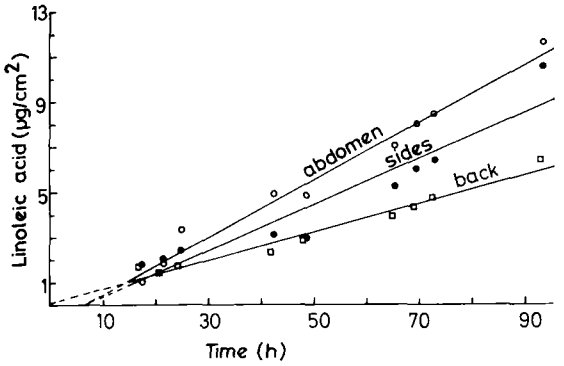

FIG. 2. Permeation of linoleic acid across hairless rat skin isolated from different areas of the animal.

greater percutaneous absorption in vivo of nitroglycerol applied on the abdominal area than on the back area of rats.

At the termination of the experiment after 95 h the amount of linoleic acid within the human skin and the rat skin was found to be 12 μg cm^{-2} and 96 μg cm^{-2}, respectively. However, this high loading, especially of the rat skin, and thereby an apparent reservoir effect of the skin, may be related to the in vitro condition. Therefore, under in vitro conditions the total skin uptake, i.e. the quantity of linoleic acid transported across the stratum cornum barrier during 95 h is a relevant parameter. The data generated in this study showed that 8·6% dose diffuses into human skin and 55·6% dose into hairless rat skin in 95 h.

When 8 μCi ^{14}C-labelled linoleic acid was applied topically to hairless rats in vivo, no radioactivity was found in the blood after 24 h. In the urine 0·3% of the radioactivity was recovered during the first 3 days after application, indicating that linoleic acid is taken up by the skin and transported into the body. In studies of the percutaneously absorbed dose fraction of fatty-acids it is necessary to count for the $^{14}CO_2$ in expired air and the radioactivity in different organs and tissues.

From the in vitro data it is seen that only about 0·9 μg linoleic acid was permeating 1 cm^2 of human skin in 24 h after application of 185 μg. However, there are certain reservations to make in the interpretation of the data with respect to the clinical situation. Firstly, the in vitro data are derived from normal skin, however, EFA deficient skin having a higher permeability to water may as well be more permeable to other substances allowing for higher penetration rate of linoleic acid. Secondly, the results of the in vitro experiments are generally lower than the permeation rate measured in vivo as pointed out by Anjo et al (1980). For a range of compounds the in vitro data may correlate but not necessarily duplicate the in vivo data. Thirdly, in the clinical situation the linoleic acid is administered in the triglyceride form by inunction of an EFA-rich oil, e.g. sunflower-seed oil (Friedman 1978). It is well-known that the chemical form of a drug influences the permeation rate, so permeation of the glyceride, a more lipophilic form, may deviate significantly.

Accounting for the above reservation it cannot be ruled out that linoleic acid is percutaneously absorbed to an extent that affects the cutaneous manifestations and the plasma biochemical abnormalities following EFA-deficiency.

However, a substitution of a daily intravenous dose of 100 mg linoleic acid is not probable. The results of this study show that free linoleic acid is a slowly permeating substance.

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