A New Application Chamber for Skin Penetration Studies *in Vivo* with Liquid Preparations

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INTRODUCTION

For the examination of skin penetration kinetics of drugs *in vivo* and vehicle effects, it is advantageous to have a system that facilitates the application of liquid vehicles on the skin for a longer period of time. Based on studies with plastic and aluminium chambers (1–4), a glass chamber is presented and validated. The chambers consist of glass to ensure a better view and to avoid drug absorption by the chamber material. This application system makes it possible to carry out penetration experiments under occlusion with the difference method. The validation of the system is performed by application of solutions containing various amounts of methyl nicotinate as model drug in polydimethylsiloxane (dimethicone) 100 to the outside of the upper arms of volunteers and measurement of the drug flux under steady-state conditions. The ANOVA results show that neither the effect of the chamber position nor the concentration effect is significant with respect to skin permeability. There is no influence of boundary layers in an *in vivo* stirring experiment.

MATERIALS AND METHODS

The application chambers (Fig. 1) are curved like the outside of the upper arms ($r = 47$ mm) of normal-weight volunteers, and they can be closed with a glass stopper. The milled ring (Fig. 2) contributes to sealing and helps to reveal early leakage of the applied liquid. Two chambers are fixed on each upper arm with a frame of spray-painted copper wire and two elastic bandages, equipped with Velcro bands (Fig. 3). The areas of application are approximately 13 cm^2, and the chamber volume varies from 2 to 5 ml.

Penetration experiments to validate the chamber system were carried out with methyl nicotinate as the model drug, dissolved in dimethicone 100 at four concentrations (0.032, 0.064, 0.096, 0.128 g/100 ml) which do not cause too strong an erythema. The solvent should not alter the barrier properties because of its high molecular weight of 6700. Twelve healthy volunteers were tested *in vivo*. After a 1-hr pretreatment with drug-free dimethicone 100 to saturate the skin and to ensure accurate determination of the drug concentration, the four chambers of each volunteer were emptied and then filled with the four solutions of different methyl nicotinate concentrations by gas-tight glass syringes, equipped with Teflon cannulas. At fixed time periods (40 min) the chambers were emptied and refilled with the four initial drug solutions. This procedure was repeated nine times. The concentration decrease in each time interval was below 10%. Thus, zero-order kinetics were achieved.

To investigate the influence of boundary layers, *in vitro and in vivo,* stirring experiments were carried out with a small stirrer (1000 rpm), attachable to the glass chambers through the opening. The *in vitro* stirring experiments were performed with a LDPE–copolymer membrane (50 μm thick), which shows drug penetration rates in a comparable order of magnitude as the skin. This copolymer membrane is easier to handle than excised human skin and does not show interactions with the donor and receptor phase.

The membrane was fixed to the chambers with screw clamps and a silicone rubber ring. In order to ensure sink conditions, 0.1 *N* HCl was used as acceptor medium, which transfers the base methyl nicotinate into the salt form. Due to the significant stirring effect *in vitro,* stirring experiments were carried out *in vivo* with six volunteers, time intervals of 60 min, and six determinations.

The concentrations of the donor-phase samples were measured spectrophotometrically, since the UV spectra of the dimethicone 100 samples obtained by the skin pretreatment did not show alterations caused by skin contaminants. This method enables the measurement of concentration decreases of even a few percent. The methyl nicotinate fluxes (mass per unit of area and time) were calculated from the concentration differences between the initial solution and the samples obtained after fixed periods of time multiplied by the volume of the respective chambers and divided by the application area and the individual time intervals.

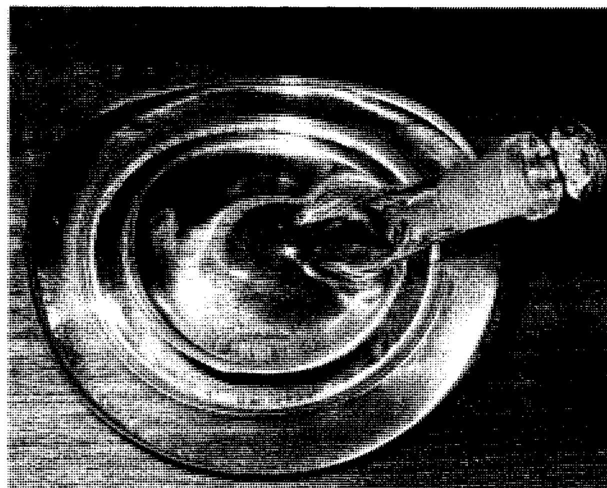

Fig. 1. Glass chamber, upper view.

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Fig. 2. Schematic representation of the glass chamber. The unit of length represented is millimeters. (a) Cross section; (b) longitudinal section.

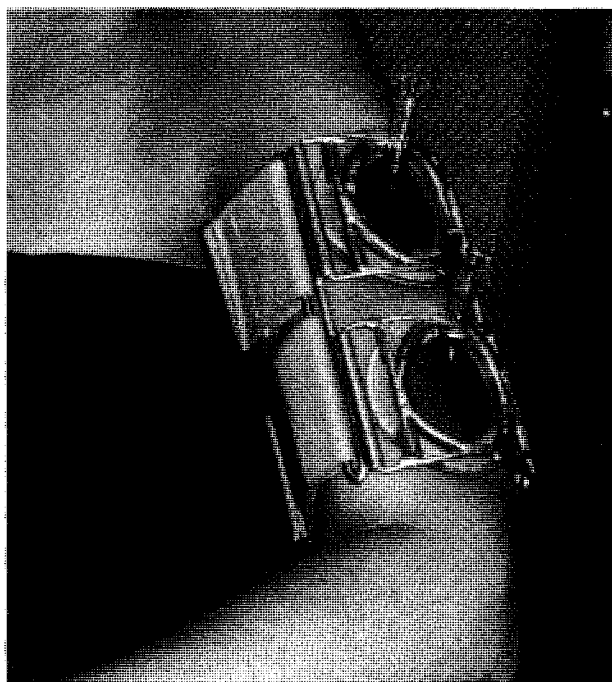

Fig. 3. The filled chambers attached to the outside of the upper arm of a volunteer.

RESULTS AND DISCUSSION

According to Fick's first law the flux should increase proportionally to the vehicle concentration. Figure 4 shows the measured steady-state flux values (mean of seven determinations) of 12 volunteers as a function of the vehicle concentration. For statistical evaluation of the *in vivo* results, these steady-state flux values (zero-order penetration rate constants per square centimeter) were divided by the corresponding concentration. These skin permeabilities P_B were calculated to investigate a concentration effect and a chamber position effect with the ANOVA of a Latin square design. There is neither a significant concentration effect nor a significant position effect ($P = 0.05$) but a significant difference exists between the permeabilities found for the 12 volunteers. The interspecimen variation is comparable with data published by Southwell *et al.* (5). Figure 5 shows the concentration-independent permeabilities of the 12 volunteers.

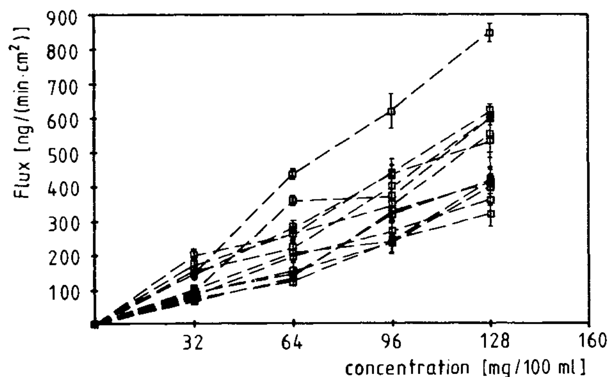

Fig. 4. Plot of methyl nicotinate fluxes from dimethicone 100 as a function of concentration; means of seven determinations ± SD (12 volunteers).

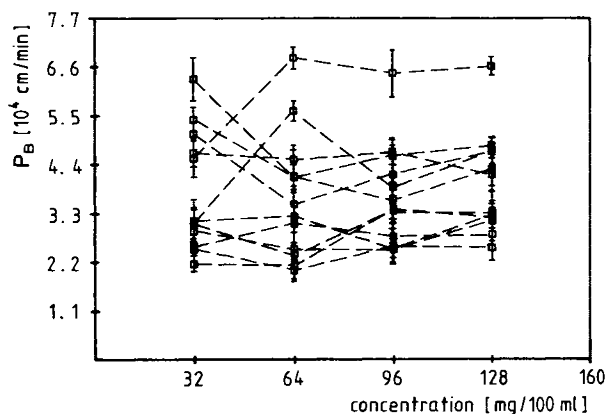

Fig. 5. Plot of permeabilities P_B versus concentration; means of seven determinations ± SD (12 volunteers).

The results of the stirring experiments are shown in Fig. 6. A significant stirring effect exists *in vitro,* interpreted as an effect of boundary layers. The results of the *in vivo* experiments with six volunteers, which were performed to see if there is a similar stirring effect detectable *in vivo,* show that an influence of boundary layers is not significant, probably due to the lower flux values *in vivo* compared with the *in vitro* results.

In summary, the study presented in this paper shows that the application system is suitable for penetration studies with liquid preparations. It makes it possible to apply liquids for over 7 hr on the upper arms of volunteers. It is possible to carry out studies under steady-state and pseudo-steady-state conditions dependent on the extent of the penetration rate constant of the used drug and the area/volume quotient of the chamber (2).

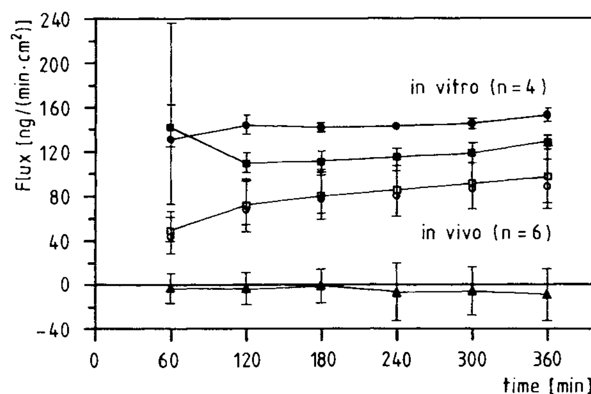

Fig. 6. Influence of stirring on the flux of a 0.032% (w/v) solution of methyl nicotinate in dimethicone 100 *in vitro* (n = 4) and *in vivo* (n = 6). Plot of fluxes versus time; means ± SD. (○, □) *In vivo* experiments; (●, ■) *in vitro* experiments (■, □) without stirring and (●, ○) with stirring; (▲) differences of flux *in vivo*.

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