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Penetration of Benzene Through Human Skin

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Although it is known that benzene may be absorbed from inhaled air, the amount that may enter the system by percutaneous absorption is less well established. We have measured the penetration of benzene through human abdominal skin in vitro from solutions in water, gasoline, hexadecane, and isooctane and found permeability constants which averaged 111.0, 1.4, 0.9, and 3.7 × 10^{-3} cm·h^{-1}, respectively. The stratum corneum/water partition coefficient for benzene has been measured and averages 30.0. The partition coefficients for the other vehicles are very low and cannot be measured by the method used for water. A new method is presented for calculating these coefficients, however, and they are 0.11, 0.14, 0.17, and 0.19 for gasoline, hexane, isooctane, and hexadecane. The flux of benzene through epidermis in vitro from air saturated with benzene at 31°C averages 1.0 μl cm^{-2}·h^{-1}.

Solvents may alter the barrier characteristics of the stratum corneum. Polar and nonpolar molecules probably traverse the stratum corneum via different pathways. By measuring the change in the permeability constants for tritiated water (a polar molecule) and for benzene (a relatively nonpolar molecule) before and after exposure to different solvents, alterations of the polar and nonpolar pathways have been shown to differ.

Since benzene penetrates normal intact human skin more rapidly than many small organic molecules, and is potentially toxic, the skin should be considered a portal of entry for benzene. Good hygiene should be maintained and care taken to avoid lengthy exposure to solvents containing benzene.

If benzene enters the system it is potentially hematotoxic and carcinogenic. For some years, industries such as the rubber, printing, and plastics industries have recognized its possible toxicity and have taken steps to reduce exposure to benzene. Snyder [1] has written a historical perspective of risks from industrial exposure to benzene.

While it has been recognized that liquid benzene, as such or in various solvents, may contact the skin and be absorbed percutaneously, the major absorption has been thought to result from inhalation. Rusch et al [2], citing earlier work by Teisinger et al, state that 46% of inhaled benzene is absorbed. If a respiratory rate of 16 per min and a tidal volume of 0.5 liters are assumed, 7.5 μl of benzene would be absorbed each hour through the lungs of a person working in an environment of 10 ppm.

Limited quantitative data are available on the penetration of benzene through the skin. Hanke et al [3] found that benzene penetrated the skin of the human forearm at the rate of 0.4 mg·cm^{-2}·h^{-1}. They also found that 10 mg of benzene were absorbed by whole-body (nude) exposure to benzene vapor for 7 h. Cesaro [4] found little evidence of percutaneous absorption by the trunk and limb of 8 subjects following exposure to benzene. Conca and Maltagliati [5] also found little evidence of absorption following 25- to 35-min exposure of their hands and forearms.

Maibach and Anjo [6] found only about 0.172 ± 0.139% of the applied dose of liquid benzene penetrated the skin of living monkeys. Lazarew et al [7] measured 138 mg of benzene in the exhaled air of rabbits held with their feet immersed in benzene for 2 h.

Most of these studies of the percutaneous absorption of benzene have been conducted in vivo in humans. Franz [8], however, studied penetration of liquid benzene in 3 species both in vivo and in vitro. When he applied 5 μl of benzene per cm^2 of skin, 0.2% or less of the applied dose penetrated. He concluded that "percutaneous absorption of benzene is lower in man than in either the mini-pig or monkey" and that "there is good overall agreement with respect to the magnitude of benzene absorption between the *in vitro* and *in vivo* sets of data." Franz recognized that a very high percentage of the applied dose that he used evaporated. When he placed larger amounts of benzene on monkey skin in vitro, he observed a penetration rate of 0.15 μl·cm^{-2}·h^{-1}. For human skin in vitro, 0.9 μl of benzene per cm^2 penetrated in about 3 h when 520 μl·cm^{-2} was applied.

In most of the in vivo experiments, the amount of benzene penetrating has been determined by observing the amount of benzene or its metabolites in the blood and/or the amount in the urine or exhaled air. Occasionally the removal of benzene from the blood by fatty tissues has been observed. The fate of benzene once it penetrates the epidermis and reaches the bloodstream is complex; it may be metabolized to various products, it may partition out of the blood and into other tissues, or it may be excreted. Therefore it is difficult to relate quantitatively the amount of benzene found in the body or excreted to the amount penetrating the skin.

For studying the penetration of benzene through the skin, we have chosen an in vitro technique. We recognize that it is not firmly established that this in vitro technique represents the in vivo situation. In the in vivo situation, the benzene which penetrates the stratum corneum diffuses quickly to the papillary dermis where it may be taken up by the bloodstream and systemically distributed. Our working hypothesis is that diffusion through the stratum corneum is the rate-limiting step, and that, since this is a passive process through nonliving tissue, it is rate-limiting both in vivo and in vitro. Benzene has some water solubility (0.2 ml per dl); were it less water-soluble, it is possible that diffusion through the stratum corneum might not be the rate-limiting step.

With in vitro techniques it is possible to investigate multiple parameters that are more difficult to investigate with in vivo techniques. The parameters that have been studied are: (1) the effect of the vehicle on the rate of penetration of benzene; (2) the effect of the vehicle on the barrier characteristics; (3) penetration of benzene from the vapor phase; (4) the partition coefficient, and (5) the diffusion constant. We have attempted to quantify the rate of penetration of benzene across human epidermis from various vehicles and from environmental air in order to be able to estimate the total amount that may enter the body through the skin.

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MATERIALS AND METHODS

Skin Preparation

Human abdominal skin obtained at autopsy was used. Epidermal sheets were separated from full-thickness skin by immersing it in water at 60°C for 30 s [9]. This separation occurred at the basal layer. From past experience we feel that this separation seldom significantly alters the barrier capacity of the stratum corneum. If the epidermis was not used at once, it was wrapped in aluminum foil and held at refrigerator temperature (4°C). When only the stratum corneum was needed, the epidermal cells could be rubbed from the stratum corneum with a moist cotton-tipped applicator if the epidermis was first floated on a trypsin solution [10]. The stratum corneum was dried over Drierite when it was used for determining partition coefficients.

Materials

We studied benzene penetration from 4 nonpolar vehicles (hexadecane, isooctane, hexane, and gasoline), from water, from air, and from liquid benzene. Gasoline, which commonly contains some benzene, is a nonpolar vehicle of considerable interest because skin exposure is not unusual in certain occupations. The benzene used had been glass-distilled and was obtained from Burdick and Jackson Laboratories; certified isooctane was obtained from Fisher Scientific Co., hexadecane, 99%, from Aldrich Chemical Co.; and tritiated water from New England Nuclear Co. The gasoline was a standardized reference sample (PS-6) obtained from the American Petroleum Institute which contained 2% benzene.

Equipment

Glass diffusion chambers and the techniques used for measuring percutaneous absorption were described by Scheuplein [11]. We used several sizes of diffusion chambers, some of which had a well. Most commonly the volumes of the donor and receptor compartments ranged from 2.0–3.0 ml. A temperature of 31°C was used, which is a reasonable average for the temperature of the cutaneous surface in vivo. The nonflowing receptor (0.1% NaCl solution) was stirred continuously with a Teflon-coated magnet. The weak sodium chloride solution was used as the receptor because we often checked for "holes" in our stratum corneum specimens by measuring its electrical conductivity.

Since benzene has a low boiling point and a high vapor pressure, it is easily lost to the atmosphere from aqueous solutions. Therefore, chambers were tightly capped at all times. Glass caps were generally used, but if a septum was needed for sampling, the rubber septum had a Teflon shield (Pierce Chemical Co., Rockford, Illinois), since benzene is very soluble in rubber.

For measuring the penetration of benzene from saturated aqueous solution or from the vapor phase and occasionally from pure benzene, the diffusion chambers used are shown diagrammatically in Fig 1. For studying the penetration of benzene from an aqueous solution, the donor chamber was filled with saturated aqueous benzene to a level above the side arm and a thin layer of pure benzene placed on top of the saturated aqueous solution. As benzene was lost from the solution by penetration through the skin and into the receptor, the stirred donor was kept continuously saturated from the benzene layer on its surface. Humans are seldom, if ever, exposed to air saturated with benzene, but we chose to study vapor transport by observing penetration from a small amount of benzene placed in the well of the donor chamber and allowed to evaporate.

A Packard Scintillation Spectrometer, Model 3330, was used for determining radioactivity of tritiated water.

Benzene Penetration

The amount of benzene in the aqueous receptor was quantified by injecting 2-μl samples of the receptor directly onto a 1.5% OV101 (liquid methyl silicone-Varian) on Chromasorb column of a Varian 2400 gas chromatograph. From a knowledge of the volume of the receptors and the concentration of benzene in the receptor at intervals following the beginning of the experiment, the flux (J_s) of benzene could be evaluated.

Passive diffusion across the stratum corneum may be expected to follow Fick's law, which states that flux of a substance is proportional to its difference in concentration on the two sides of the membrane:

$$J_s = k_p \Delta C_s \qquad (1)$$

where J_s = flux, ΔC_s = difference in concentration, and k_p = permeability constant.

The permeability constant may be thought of as flux normalized for concentration. In vivo, any benzene that penetrates the stratum corneum may be expected to diffuse through the viable epidermis and enter the capillaries in the papillary dermis. Benzene and other molecules diffuse much more rapidly through the epidermis than through the compact stratum corneum and are subsequently removed from the skin by the blood flow. Thus, the concentration of benzene in the epidermis will be very low. In in vitro experiments the concentration of the benzene in the fluid on the receptor side of the chamber is kept very low compared to its concentration in the solution on the donor side. In our calculations, ΔC_s has been equated to the donor concentration.

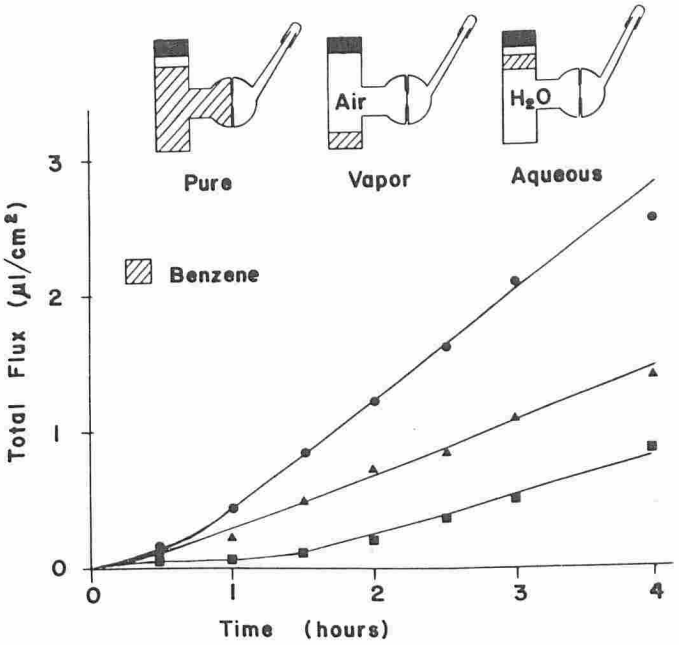

FIG 1. Flux of benzene from pure benzene ●, from benzene vapor ▲, and from water saturated with benzene ■ through human epidermis into 0.1% saline solution.

In this paper, we treat the concentration gradient of benzene across the stratum corneum as the driving force, even though the flux of benzene is driven by the gradient in chemical activity across this tissue. We are assuming that the internal and external surface layers of the stratum corneum have the same activity coefficient for benzene, and that, since the system is closed and the receptor is always water, both layers are equally hydrated at equilibrium.

The permeability constant may be meaningfully expanded as follows:

$$k_p = \frac{K_m D_m}{\delta} \qquad (2)$$

where K_m = partition coefficient, which is

$$\frac{\text{solubility in stratum corneum}}{\text{solubility in vehicle (donor)}},$$

D_m = diffusion constant, and δ = membrane (stratum corneum) thickness.

Partition coefficients were determined by a method described by Scheuplein [11] in which a known weight of dry stratum corneum is allowed to come to equilibrium with a measured volume of weak solution of benzene. The concentrations of benzene in the solution before the stratum corneum is added and after equilibrium has been reached are determined. From the difference in concentrations, the amount taken up by the stratum corneum is calculated. It is important that the difference in concentration represents only benzene entering the stratum corneum; loss by evaporation or solution into a rubber septum must be avoided. Instead of the container used by Scheuplein, we used 0.2-ml microvials, obtained from Pierce Chemical Co., with Teflon-shielded rubber septums. The vials were completely filled so that no benzene evaporated into any head space. The Teflon shield was punctured for sampling only once at the end of the experiment. This technique may be used for aqueous solutions of benzene for which the partition coefficient is high but not for solutions of benzene in

hydrocarbons for which partition coefficients are very low. We believe, however, that such coefficients can be calculated by a method described in *Results*.

The exact thickness of the stratum corneum for each piece of skin is difficult to determine experimentally but the overall range of thickness is known and small errors in this parameter will not significantly influence overall conclusions. Fully hydrated stratum corneum is considerably thicker than dry stratum corneum.

These equations assume that neither the penetrant nor the vehicle alters the barrier capacity of the stratum corneum. Unfortunately, this is rarely true: water, for instance, changes its thickness; many vehicles may delipidize the tissue. These changes may affect not only the barrier capacity but also the partition coefficient.

Possible alterations in the barrier capacity of the stratum corneum were determined by observing the permeability constants for benzene (nonpolar) and tritiated water (polar), before and after contact with various vehicles. Penetration rates of these two substances were measured simultaneously from a saturated aqueous solution of benzene to which a trace of tritiated water was added. Permeability constants could be obtained in 3 h, then the donor and receptor fluids removed, a vehicle kept in contact with the stratum corneum for the desired period, removed, and permeability constants for benzene and tritiated water again determined. This method measures barrier characteristics of strongly hydrated stratum corneum since penetration is measured with water in both donor and receptor.

RESULTS

Flux

Fig 1 shows the flux of benzene from pure benzene, from air saturated with benzene vapor, and from a saturated aqueous solution through human epidermis into 0.1% saline solution. Note that steady-state flux is quickly established after short lag periods. There is no indication of any membrane alteration which causes any significant change in the slopes of the curves. From multiple experiments of this type, the fluxes of benzene from these 3 systems are: pure benzene 2.11 ± 1.08, benzene vapor 1.04 ± 0.37, and aqueous solution 0.22 ± 0.05 $\mu l \cdot cm^{-2} \cdot h^{-1}$.

Vehicles

From flux measurements, the permeability constants ($cm \cdot h^{-1}$) for benzene, penetrating from hexadecane, isooctane, and gasoline, each 5% (v/v) solutions, and from a saturated aqueous solution, through epidermal samples into 0.1% saline, can be calculated and are shown in Table I. For the hydrocarbon vehicles, penetration is more rapid from isooctane than from hexadecane. When used as a vehicle, gasoline seems to function more similarly to hexadecane than to isooctane. Standard deviations are somewhat large. More experiments might change the means and decrease the SD somewhat, but we do not believe the general conclusions would be different.

TABLE I. *Permeability constants[a] ($k_p \times 10^3$ $cm \cdot h^{-1}$) for benzene penetrating from four vehicles*

	Hexadecane	Isooctane	Gasoline	Water
		4.4, 4.2, 4.0[b]		152
		4.9, 4.9		141
		5.3, 4.2		115
			1.7	83
	1.5			133
	0.5, 1.4	3.1, 2.2		141
	1.4, 1.6	2.4, 1.4		94
			1.2, 2.8	111
	0.8, 1.1		1.0, 1.3	82
	0.6		1.4	84
	0.5, 0.6			131
	0.8		1.0	93
	0.8			84
	0.7, 0.8		0.9, 1.1	
Mean ±SD	0.94 ± 0.38	3.73 ± 1.26	1.40 ± 0.58	111.1 ± 25.9

[a] Flux normalized for concentration.

[b] For the hydrocarbon vehicles only, all samples on a single horizontal line were from a single specimen of skin.

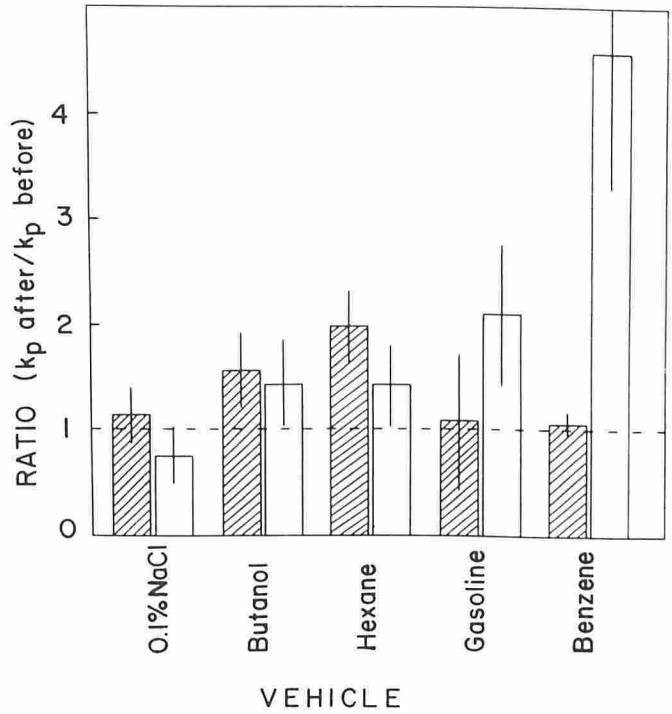

FIG 2. Change in permeability constants (k_p) for benzene (▨) and tritiated water (□) after 3-h contact with various vehicles. *Error bars* are SD.

It is seen that the permeability constant for benzene from various vehicles differs. Permeability is strongly dependent on partition coefficient, which varies significantly for different vehicles. Also, the vehicle may alter the barrier characteristics of the stratum corneum.

Alterations of the Barrier Characteristics of the Skin

Fig 2 shows the ratios of the permeability constants for benzene and tritiated water taken before and after 3-h contact with various vehicles. A ratio of 1 signifies no change in barrier capacity following vehicle contact. A very weak salt solution caused little or no change in the permeability constants of either molecule; butanol and hexane make the skin somewhat more permeable to both molecules; gasoline and benzene make the skin more permeable to water but not to benzene. Hence, it is seen that different vehicles may alter the polar and nonpolar pathways differently.

Partition Coefficients for Benzene in Polar and Nonpolar Vehicles

As we measured the partition coefficient for benzene between water and hydrated stratum corneum, we found variation from 20 to 35 with a mean of 30. The partition coefficient between hexadecane and stratum corneum is low and cannot be satisfactorily determined by the method described. However, with the stratum corneum/water partition coefficient known, the stratum corneum/hexadecane partition coefficient can be calculated in the following manner.

In measuring the penetration of benzene from a 5% solution in hexadecane through epidermis into 0.1% NaCl solution, penetration occurs rapidly; steady state flux is usually reached during the first hour. However, as the concentration of benzene in the receptor continues to increase, the *rate* of penetration decreases until equilibrium is reached, after which time the concentration of benzene in the receptor remains constant. This equilibrium is reached at a receptor concentration less than saturation for an aqueous solution ($2 \mu l \cdot ml^{-1}$). The receptor concentration at equilibrium is identical to the concentration of benzene in water which has been allowed to come to

TABLE II. *Penetration of benzene through epidermis from various vehicles*

	Vehicle					
	Water	Hexadecane	Isooctane	Hexane	Gasoline	
Donor concentration, C_d $\mu l \cdot ml^{-1}$	2	50	50	50	50	Experimental
Flux, J_s $\mu l \cdot cm^{-2} \cdot h^{-1}$	0.22	0.05	0.19	0.12	0.07	Experimental
Permeability constant $k_p \times 10^3$ cm·h^{-1}	111	0.94	3.73	2.4	1.4	$k_p = J_s/C_d$
Partition coefficient, K_m	30	0.19	0.17	0.14	0.11	Experimental and calculated
Thickness δ μm	40	40	40	40	40	Estimated
Diffusion coefficient $D_m \times 10^9$ cm^2·s^{-1}	4.1	5.5	24.4	19.0	14.0	$D_m = k_p\delta/K_m$

equilibrium with the donor without the presence of the skin. For 5% benzene in hexadecane, the aqueous concentration at equilibrium is 0.31 $\mu l \cdot ml^{-1}$.

At equilibrium, the concentrations of benzene in the stratum corneum at the donor boundary and at the receptor boundary are identical, since if they were different a net flux would result and at equilibrium there is no net flux. The concentration in the stratum corneum at the receptor boundary is the concentration in the receptor multiplied by the stratum corneum/water partition coefficient. For this system, it is 0.31 $\mu l \cdot ml^{-1} \times 30 = 9.3$ $\mu l \cdot ml^{-1}$, and we have said that this must be the concentration in the tissue at the donor boundary also. The concentration of benzene in the hexadecane donor is 50 $\mu l \cdot ml^{-1}$ and thus the stratum corneum/hexadecane partition coefficient is 9.3/50 = 0.19.

Using the same method, it is possible to determine the stratum corneum/solvent partition coefficient for any solvent immiscible with water. Partition coefficients for benzene in isooctane, hexane, and gasoline are 0.17, 0.14, and 0.11, respectively. The stratum corneum is a relatively good "solvent" for benzene; it is much better than water but not as good as many nonpolar, organic liquids.

Diffusion Constant

Diffusion constants of molecules moving across the stratum corneum are not measured directly. If lag time and thickness are known, diffusion constants can be calculated. We have found, however, that lag times are difficult to determine accurately. We chose to calculate diffusion constants from a knowledge of permeability constants, partition coefficients, and estimated thickness (see Eq. 2). We believe that the aqueous receptor strongly hydrates the stratum corneum in the diffusion chambers and that 40 μm is a reasonable estimate of its thickness. Table II is a summary table showing these various parameters for benzene penetrating human epidermis from 5 different vehicles and entering a 0.1% saline solution on the receptor side. Diffusion constants for benzene, penetrating from hydrocarbon solvents, vary from 4.1 to 24.4 $\times 10^{-9}$ cm^2·s^{-1}. These are all high compared to the diffusion constant for water in stratum corneum, which is 5×10^{-10} cm^2·s^{-1} [11]. The vehicles will enter the stratum corneum as well as the benzene, and this may account for some of the differences in diffusion constants for benzene when presented to the skin in different vehicles.

DISCUSSION

The solubility characteristics of benzene are such that it is easily taken up by the stratum corneum. Once in the stratum corneum, it does not meet many restraining forces to impede its movement and diffuses easily. The permeability constant for benzene, as determined in vitro, is higher than that of many other small molecules, particularly those having one or more polar groups.

Since accurate quantitative permeability data are difficult to obtain in vivo, it is tempting to apply in vitro data to in vivo situations. We recognize that differences exist in the two systems, particularly with respect to hydration of the stratum corneum. Our in vitro closed system is more closely matched by an in vivo system in which there is occlusion. There are occupations in which some areas of the skin, such as the hands, may be covered for some time with an occlusive oily film. More quantitative data are required in order to know the effect of hydration of the stratum corneum and the effect of alteration of the in vivo water concentration profile on the penetration of benzene.

Even though these uncertainties exist, and more data are needed to support the Franz conclusion that there is good overall agreement between in vitro and in vivo data [8], we have chosen to calculate from our in vitro data and from inhalation data of others the amount of benzene entering the body under specific environmental conditions. An adult working in ambient air containing 10 ppm of benzene, with 100 cm^2 of glabrous skin in contact with gasoline containing 5% benzene, and his entire skin (2 m^2) in contact with ambient air, will absorb in an hour, 7.5 μl of benzene from inhalation, 7.0 μl from contact with gasoline, and 1.5 μl from body exposure to ambient air. Since our in vitro techniques measure the penetration of benzene through strongly hydrated stratum corneum, the calculated flux may be higher than under some in vivo conditions. Nevertheless, it seems that unless good hygiene is maintained and care is taken to prevent lengthy exposure to solvents containing benzene, significant amounts of benzene may enter the body through the skin.

We have not addressed the fate of the benzene once it penetrates the stratum corneum and thus, from this study alone, the potential toxicity of benzene that has entered through the skin cannot be determined. It seems apparent, however, that if one is concerned about the systemic effects of benzene, contact of the skin with benzene and solvents containing benzene should be avoided as much as possible.

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