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CHAPTER 5

PERCUTANEOUS ABSORPTION OF BENZENE

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ABSTRACT

A critical factor in the evaluation of the risk associated with cutaneous exposure to toxic substances is information relating to the percutaneous absorption of those substances. Widespread exposure to benzene in the industrial environment and knowledge of its carcinogenicity has prompted an evaluation of its absorption through the skin.

The percutaneous absorption of benzene has been measured in the monkey, the miniature pig, and man using both *in vitro* and *in vivo* techniques. Following the application of a thin layer (5-10 μl/cm²) of benzene *in vivo*, absorption was found to average less than 0.2% of the applied dose in all species studied. Under the conditions of these experiments, the remainder of the applied material quickly volatized and was lost to the atmosphere. Total absorption was 0.14% in the monkey, 0.09% in the mini-pig, and 0.05% in man. Peak excretion of radioactivity occurred in the first two hours and decreased rapidly thereafter. *In vitro* studies demonstrated rapid penetration of benzene through the skin. When the same dose applied *in vivo* was used *in vitro*, the peak rate of absorption occurred at 15-40 minutes and total absorption was similar to that measured *in vivo*. Absorption was 0.19% in the monkey, 0.23% in the mini-pig, and 0.10% in man. Benzene absorption was found to be a function of its contact time with the skin. Application of progressively larger doses which persisted on the skin for up to 3 hours resulted in 10-100 times greater absorption. Total absorption was found to be directly related to the length of time benzene remained on the skin.

It can be concluded that a major factor controlling the percutaneous absorption of benzene is its contact time with the skin. Under ideal conditions in which the contact time is short due to its inherent volatility, less than 0.2% of the applied dose will be absorbed, or approximately 0.01 μl/cm². The *in vitro* technique used in this study appears to be a valid means by which to assess the percutaneous absorption of benzene.

INTRODUCTION

There is increasing concern over the skin as a route of entry into the body for toxic substances, particularly in the work environment where the number of potentially harmful substances is large and exposure may be of long duration. Whereas the skin had previously been thought to be a relatively impervious barrier, more recent work has clearly shown that it is a barrier of variable permeability (1). Though many substances penetrate poorly, if at all, others are relatively well-absorbed. Indeed, it has been shown that the skin is quite permeable to simple organic compounds such as benzoic acid and dinitrochlorobenzene, with approximately 25%-50% of the applied dose being absorbed in 24 hours (2, 3). Thus, a critical factor in the evaluation of the risk associated with cutaneous exposure to toxic substances is information relating to their percutaneous absorption. In this study, the percutaneous absorption of benzene, a known carcinogen widely used in industry, has been measured in animals and man using both *in vitro* and *in vivo* techniques.

MATERIALS AND METHODS

Radioisotopes

All studies were conducted using ^{14}C-benzene, 40 mCi/mmole (New England Nuclear Corporation, Boston, MA). Prior to use, it was diluted with reagent-grade, unlabelled benzene (Mallinkrodt) to reduce its specific activity to a workable range.

In Vivo Experiments

Animal Studies

Three adult rhesus monkeys of each sex and one mini-pig of the improved Pitman-Moore strain (VitaVet Laboratories, Inc. Marion, IN) of each sex were used to assess benzene absorption.

The basic procedure was the same in both cases. At least 24 hours prior to an experiment, the animal's back was shaved with electric clippers; it was then placed in a metabolic chair (monkey) or cage (mini-pig) for the collection of control urine specimens. Subsequently, 0.5 ml of benzene containing 100 μCi ^{14}C-benzene was applied to the back and allowed to flow over the skin, seeking its own area. Application was made with the animal positioned in front of a fume hood so that as the material volatized, none was inhaled.

There was no way to assure that the rate of evaporation was the same for each animal under these conditions. However, in all cases, the applied dose was not visible after 30 seconds. In a separate series of experiments, the dye oil red O was incorporated into nonradioactive benzene and applied to the back, under the same experimental conditions, to allow measurement of the application area. It was found to vary from 55-75 cm^2.

All urinary excretion was collected over the next 2-4 days and analyzed for radioactive content. A 5 ml aliquot of urine was gelled by the addition of 10 ml Aquasol-2 (New England Nuclear Corporation, Boston, MA) counted in a Beckman liquid scintillation spectrometer, and corrected for quenching by the external standard method. To ensure obtaining frequent urine specimens at early times in the monkey experiments, the animals were given fruit immediately before and after benzene application. If an animal did not void within the first 90 minutes, an intramuscular injection of 1 mg/kg furosemide was given. This was not done with the mini-pigs.

In order to properly quantitate percutaneous absorption from urinary excretion data, it is necessary to know what fraction of an absorbed dose is excreted in the urine since this is only one of several possible excretory pathways available to benzene. This factor was determined in the rhesus monkey by means of a subcutaneous injection of benzene. In a separate series of experiments, approximately 0.05 ml radioactive benzene was injected into the subcutaneous space of the back. As before, the animal was confined to a metabolic chair and all urinary excretion collected and assayed for radioactive content. Special care was taken to ensure that none of the injected material leaked back through the needle track as this could result in an underestimation of the fraction excreted in the urine. Leakage was most easily detected by the positioning of a Geiger-Muller tube adjacent to the injection site. Only radioactive material which had leaked onto the surface of the skin would register on the detector, as the thickness of the skin itself was sufficient to screen out radioactivity in the subcutaneous space.

Human Studies

Four adult males in good health and without evidence of skin disease were used to measure benzene absorption. With the volunteer seated in front of a fume hood and wearing a respirator suitable for trapping organic vapors, 0.4 ml benzene containing 100 μCi ^{14}C-benzene was applied to 80 cm^2 of ventral forearm. All urine was collected and analyzed for radioactive content until background levels of activity were reached. An aliquot of each urine specimen was assayed following the same procedure used in the animal experiments. All data were corrected for incomplete urinary excretion using the factor determined from the monkey subcutaneous experiments.

In Vitro Experiments

Benzene absorption *in vitro* was measured using a previously published method which had been shown to give results correlating well with those obtained *in vivo* (3, 4). Skin from man, monkey, and mini-pig was used in these experiments so that comparison to the *in vivo* could be made. All skin was obtained at autopsy. (Human abdominal skin was obtained from local hospitals. Back skin from *Macaca menestrina* monkeys was supplied by the University of Washington Regional Primate Center (supported by Grant

#RR00166 from the National Institutes of Health), and back skin from VitaVet mini-pigs was obtained from animals sacrificed in our own laboratory.)

Essentially, the method consists of mounting freshly obtained split thickness skin between two halves of specially contructed diffusion chambers. The dermis is bathed by isotonic saline, pH 7.4, 37°C, and stirred by a teflon-covered magnet which is driven by an externally mounted 600 RPM timing motor. The epidermis is exposed to ambient laboratory conditions (21°-22°C, 35%-50% relative humidity). Following a 1-2 hour equilibration period, radioactive benzene is pipetted onto the epidermis by micropipette and the rate of absorption measured by serially sampling the dermal bathing solution. At each sampling time, the dermal bathing solution is removed in its entirety, replaced with fresh solution, and a 3 ml aliquot gelled by the addition of 4 ml Aquasol-2 and assayed by liquid scintillation counting.

RESULTS AND DISCUSSION

In Vivo Studies

Data showing the excretion of radioactivity in the urine following application of ^{14}C-benzene to the skin of man are given in Figure 1. It can be noted that in each case, the rate of excretion is greatest in the first or second collection period, occurring at 2 hours or less in three of the four volunteers, and declines rapidly thereafter. Though detectable amounts of activity are present in the urine for up to 36 hours, more than 80% of the total excretion occurs in the first 8 hours. After that time, the rate of excretion falls to very low levels which

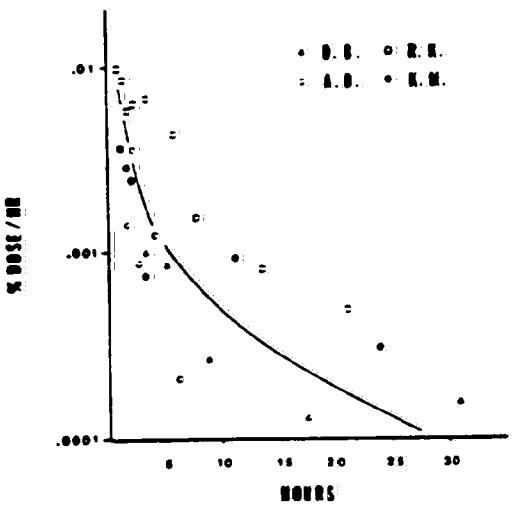

FIGURE 1. Urinary excretion of radioactivity following application of 0.4 ml ^{14}C-benzene to the ventral forearm of 4 human subjects.

are difficult to accurately measure because the amount of radioactivity present in each sample so closely approximates background. Total excretion of radioactivity in the four volunteers studied was found to be 0.023 ± 0.022% of the applied dose (Table 1).

The rate of excretion of radioactivity obtained in both the mini-pig and monkey was qualitatively similar to that seen in man. That is, the first two samples always contained the highest counts. In the mini-pig, however, the lack of an adequate number of urine specimens in the first 12 hours made meaningful comparisons of the kinetics of excretion virtually impossible. The results obtained in the rhesus monkey are given in Figure 2.

Although the overall time course of excretion is similar to that seen in man, a significant quantitative difference between the two exists. Total excretion of radioactivity was found to be three times higher in the monkey than in man with a mean value of 0.065 ± 0.037% of the applied dose. The comparable value determined in the mini-pig was 0.042% ± 0.017% (Table 1). Using the Student's *t* test, the mean urinary excretion in the monkey was found to differ significantly ($p = 0.05$) from that observed in man.

From the data on the urinary excretion of radioactivity, the percutaneous absorption of benzene can be calculated. To do this, it is necessary to know only what percentage of systemically absorbed benzene is excreted in the urine. This figure has been experimentally determined following subcutaneous injection of ^{14}C-benzene in the rhesus monkey and found to be 43.3 ± 8.9% (mean ± standard deviation of 6 animals). Therefore, the values obtained for the urinary excretion of radioactivity when multiplied by the factor to correct for excretion via other routes (100/45.3 = 2.2) give the amount of benzene absorbed through the skin following topical application. These calculated values are listed in Table 2 with the correction factor determined in the monkey used to adjust the mini-pig and human data as well.

TABLE 1. Urinary Recovery of Radioactivity Following Topical Application of ^{14}C-Benzene.

Monkey		Mini-Pig		Man	
Subject	% of dose	Subject	% of dose	Subject	% of dose
13-6, M	0.048	M-1	0.030	DB	0.008
14-6, M	0.046	F-2	0.054	AD	0.006
15-6, M	0.033			KM	0.025
X-23, F	0.076			RK	0.054
X-25, F	0.135				
X-26, F	0.052				
Mean ± SD	0.065 ± 0.037		0.042 ± 0.017		0.023 ± 0.022

It can be seen from these data that the percutaneous absorption of benzene is quite low, ranging from 0.05% of the dose in man to 0.14% of the dose in the rhesus monkey. The vast majority of the applied benzene, approximately 99.9%, apparently volatilizes to the atmosphere under the experimental conditions utilized in this study. It was noted by direct observation that liquid benzene was undetectable on the skin surface within 30 seconds of its application. Thus, because of its high volatility, a critical factor controlling the percutaneous absorption of benzene is its contact time with the skin.

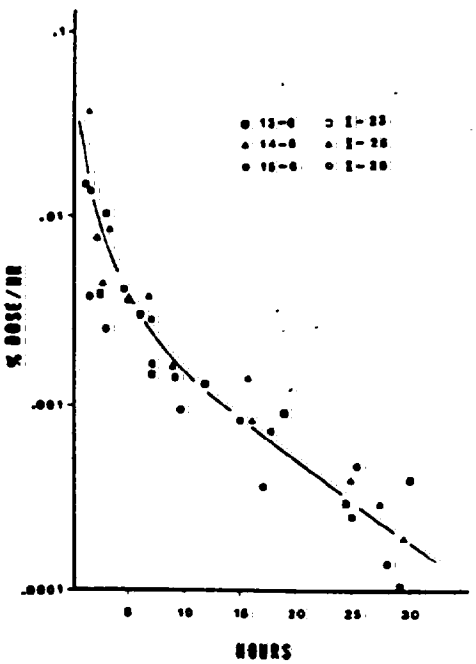

FIGURE 2. Urinary excretion of radioactivity following application of 0.5 ml ^{14}C-benzene to the shaved backs of 3 male (solid) and 3 female (open) rhesus monkeys.

TABLE 2. Benzene Absorption (% of dose[1]).

	In Vivo	Number of Experiments	*In Vitro*	Number of Experiments
Monkey	0.14 ± 0.08	6	0.19 ± 10	3
Mini-Pig	0.09 ± 0.04	2	0.23 ± 0.10	5
Man	0.05 ± 0.05	4	0.10 ± 0.004	9

[1]Dose = 5 μl/cm²

In Vitro Studies

In order to examine the relationship between benzene absorption and skin contact time and, also, to more closely define the kinetics of absorption, *in vitro* studies were conducted. A representative experiment is shown in Figure 3. Three pieces of skin from the same monkey were exposed to varying doses of ^{14}C-benzene, 5 μl/cm^2, 10 μl/cm^2, and 200 μl/cm^2.

Several things should be noted from this data. Diffusion of benzene through the skin is very rapid with the peak rate of absorption occurring at 15-20 minutes. The lag time is so short that within minutes, measurable radioactivity can be found in the dermal bathing solution. To date, of the limited compounds whose percutaneous absorption has been studied, no compound has been found that permeates the skin as rapidly as benzene (2, 3, 5, 6).

At the lowest dose (5 μl/cm^2) which corresponds to that used in the *in vivo* experiments, the rate of absorption rises to a maximum at approximately 20 minutes, then rapidly falls off over the next 2 hours. Though the rate of absorption is quite low after 3 hours, measurable absorption continues for at least 24 hours. This time course correlates well with that observed *in vivo* and suggests that part, if not all, of the long tail on the rate of excretion curve reflects continued percutaneous absorption of benzene rather than delayed excretion of material previously stored or bound within the body. The rate of absorption profile determined *in vitro* for both the mini-pig and man is similar to that seen in the monkey, although the maximum rate of absorption occurs somewhat later, generally 20-40 minutes.

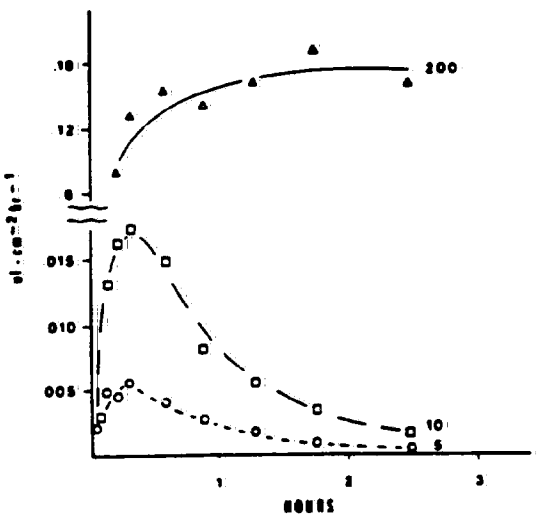

FIGURE 3. Rate of absorption of ^{14}C-benzene *in vitro* following application of 5, 10, or 200 μl cm^2 to monkey skin

Total absorption varies significantly among the three species studied, and the data for the 5 μl/cm² dose are given in Table 2. Percutaneous absorption of benzene is lower in man than in either the mini-pig or monkey; this is consistent with the observations made *in vivo*. Given the limited number of experiments, there is reasonable agreement between the *in vitro* and *in vivo* sets of data, and it appears as if the *in vitro* approach may offer a valid method for the quantitation of benzene absorption.

Benzene absorption increases as the size of the applied dose increases (Figure 3). From the laws of diffusion, this is to be expected. It can be seen that at the highest dose, a steady state is achieved, that is, the rate of absorption becomes constant. Failure to reach a steady state at lower doses is due to the rapid volatilization of benzene from the skin surface and, therefore, the absence of a source to continually support the absorption process. At higher doses in which a layer of liquid benzene can be maintained on the skin for several hours, the steady state is always observed. The relationship between dose and benzene absorption has been carefully evaluated in human skin and the data are given in Table 3 and plotted in Figure 4.

As can be noted from the figure, the relationship between dose and absorption appears to be linear. This is largely the result of the rapidity with which benzene diffuses through the skin and attains the steady state. Once the steady state is attained and the rate of absorption is constant, the amount of benzene absorbed can only increase in direct proportion to the length of time it remains on the skin. An exception to this is possible if benzene were capable of damaging the skin. Under those circumstances prolonged exposure could lead to increasing skin damage and result in an increasing rate of benzene absorption with time. However, the present data do not support this possibility.

Although the use of an increasing benzene dose is a convenient tool that can be used under *in vitro* conditions to study benzene absorption, it may be of limited toxicologic relevance as such. In an *in vitro* experiment, the dose/cm²

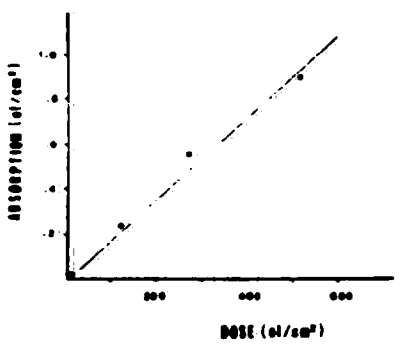

FIGURE 4. Total absorption of benzene *in vitro* in human skin following application of 5, 120, 270 or 520 μl/cm².

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FIGURE

TABLE 3. Benzene Absorption (μl/cm²) *In Vitro*: Human Skin.

Skin	5 μl/cm²	120 μl/cm²	270 μl/cm²	520 μl/cm²
1	0.018	0.156	—	—
2	0.012	0.264	0.486	1.01
3	0.007	0.264	—	1.09
4	0.010	—	—	—
5	0.009	—	—	—
6	0.007	0.201	—	0.783
7	0.008	0.241	—	0.557
8	0.014	0.232	0.513	1.08
9	0.006	0.236	0.478	0.899
	—	0.297	0.751	—
X ± SD	0.010 ± 0.004	0.236 ± 0.043	0.557 ± 0.130	0.903 ± 0.206

can be increased readily by adding more benzene to the chamber in contact with the epidermis. This is less likely to occur in the industrial setting since, under conditions in which a worker is exposed through spill or splash, the excess would run off or be absorbed by clothing. Only in a situation where there is direct immersion of some body part in benzene would the *in vitro* conditions used here be completely relevant.

A parameter which would appear to have more utility than dose is contact or exposure time. The exposure times, *i.e.*, the time to complete evaporation, in the human *in vitro* experiments reported in Table 3 were observed and noted at each dose level. The relationship between exposure time and absorption is presented in Figure 5. It can be noted that, just as the relationship between dose

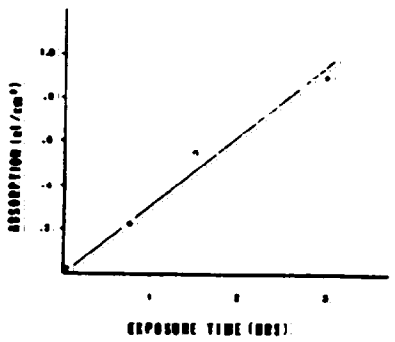

FIGURE 5. Total absorption of benzene *in vitro* in human skin as a function of the time benzene remained on the skin.

and absorption appears to be linear, the relationship between exposure time and absorption is also linear. This observation has great practical importance since it suggests that it may be possible to estimate benzene absorption in an industrial environment from knowledge of the area of skin exposed and the exposure time.

Further work is needed to substantiate this relationship and also to define the role of the vehicle (solvents or mixtures other than pure benzene) in controlling the percutaneous absorption of benzene.

ACKNOWLEDGMENT

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