PENETRATION OF LOW-MOLECULAR-WEIGHT ALCOHOLS INTO SKIN

I. Effect of Concentration of Alcohol and Type of Vehicle*

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Some of the factors which determine the rate of penetration of a neutral compound into and through normal skin are: (*a*) concentration of the penetrant in the presenting vehicle; (*b*) size of the penetrating molecule; (*c*) solubility characteristics of the penetrant; (*d*) solvent characteristics of the vehicle, and (*e*) temperature. The effect of these factors can be determined only if a good method is available for studying rates of penetration.

Ainsworth (1) and Blank (2) have reviewed the methods used for measuring permeability of the skin. Since skin is an effective barrier against the passage of most substances, no method can be satisfactory unless it permits quantitative measurement of microamounts of the penetrant. The question as to whether permeability is the same in excised skin and in the intact skin of living man remains unanswered. It is pertinent, however, that the data reported by Ainsworth (1) for three species of animals show no consistent difference between the *in vivo* and the *in vitro* rates of penetration of tri-butyl phosphate. All of the data to be presented here were obtained from excised skin.

We have chosen to study the penetration of low-molecular-weight, primary alcohols because these compounds constitute a homologous series of neutral substances of small molecular size whose solubility characteristics differ. With gas chromatography as little as 0.001–0.01 μg of these alcohols can be quantitatively measured simply and with accuracy.

METHODS

For the experiments reported here, abdominal skin was obtained at autopsy, usually within the first 12 hours after death. Excessively hairy skin was rejected. All subcutaneous fat was promptly removed. Slightly hairy skin was clipped closely, but with care to avoid injuring the skin. The electrical conductivity of each specimen was measured before proceeding further with an experiment, since it was felt that high conductivity would indicate that the skin had been damaged. All skin in which the conductivity was high was rejected.

Discs of full-thickness skin were placed on diffusion chambers such as that shown in Fig. 1. In this chamber† penetration occurs through a 3 cm^2 area of skin. The volume of the space above the skin is approximately 8 ml; that below the skin approximately 3 ml. Fig. 2 shows four of these chambers assembled in parallel. In the experiments reported here, the receptor fluid on the dermal side of the skin was normal saline solution (0.85% NaCl) that flowed through the chamber continuously at 2–3 ml/hr and was collected in test tubes in a fraction collector.

Temperature may be controlled by a water bath surrounding the chambers, but in these experiments the effect of temperature on penetration of the alcohols was not studied and all data were obtained at the temperature (22° C ± 2° C) of an air-conditioned room.

Because high-molecular-weight alcohols are poorly soluble in water, their penetration can be measured from weak solutions only. So that the concentrations of these alcohols in the receptor fluid would be high enough to permit analysis, the chambers were so modified that a small, stirred, constant-volume receptor might be used instead of a flowing receptor.

The aqueous solutions of alcohols were analyzed on a Wilkins Hy-FI Gas Chromatograph #600 with a flame detector. The column consisted of five feet of ⅛″ copper tubing with a packing of 20% glycerine on acid-washed 80/100 Chromasorb W. The oven temperature was between 60 and 75° C, and the rate of nitrogen flow was 20–25 ml/min. The apparatus included a Sargent recorder, model SR, equipped with a disc integrator so that the areas of the tracings could be read directly.

A homologous series of primary alcohols from methanol (CH_3OH) through octanol ($C_8H_{17}OH$) was studied. The concentration of an alcohol used was at times limited by its solubility in a vehicle. The vehicles used varied from polar liquids, such as water and the glycols, to nonpolar (lipid-like) liquids, such as olive oil, mineral oil and isopropyl palmitate.

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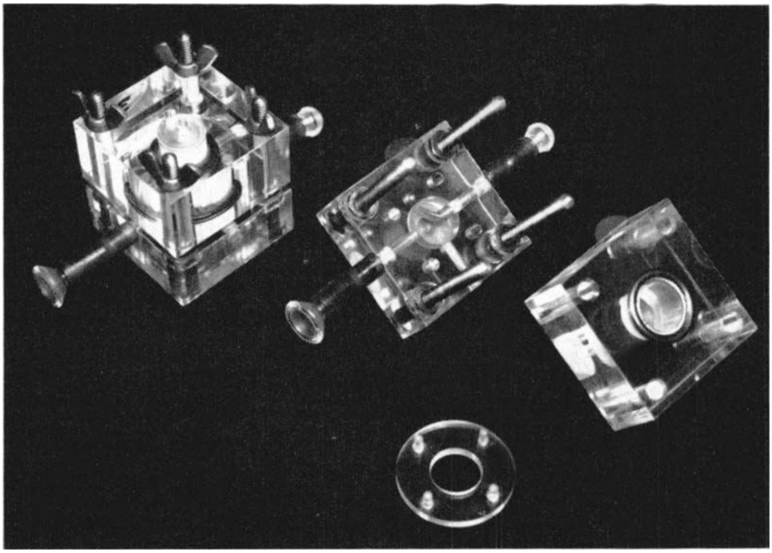

FIG. 1. Chamber for studying penetration of the skin

Records were kept of the sex and age of the subject from whom the skin was taken. Since all of the skin was obtained at autopsy, the majority of specimens were from elderly subjects. No attempt has been made to correlate penetration rates with age or sex.

The results of the experiments are expressed as flux (J_s), *i.e.*, the amount of material (micromoles) that penetrates a unit area of skin (1 cm^2) in unit time (1 hr), or as permeability constant (k_p). The relationship of the flux to the permeability constant (Fick's Law) is shown in the following equation:

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

where ΔC_s is the difference in concentration of the the penetrant on the two sides of the skin. In the system which has been described, ΔC_s may be considered to be equal to the concentration of the penetrant in the vehicle, since the concentration in the receptor on the dermal side is low and in most instances the receptor is continuously flowing. If J_s is expressed as $\mu M \ cm^{-2} \cdot hr^{-1}$ and ΔC_s as $\mu M \cdot cm^{-3}$, then the units of k_p are $cm \cdot hr^{-1}$. All experiments were run for at least 24 hours. Steady state was usually reached during this period; when it was not, a notation is made to that effect.

RESULTS

1. *Concentration*

The relationship between concentration of the penetrant and flux for butanol and pentanol as they penetrate from normal saline solutions is shown in Fig. 3. There is a small deviation from the straight-line function predicted by Equation 1, but for low concentrations this deviation is not great.

As pentanol penetrated from a 0.2 M saline solution, flux varied from 0.89 to 1.31 $\mu M \ cm^{-2} \cdot hr^{-1}$ (mean 1.18; S.D. 0.19) for the skin of 10 different subjects. This degree of variation is probably attributable to biological differences in the various samples of skin and to unrecognized variations in technic.

2. *Molecular Weight*

Pentanol penetrates more rapidly than butanol (Fig. 3); in other words, the compound with the higher molecular weight penetrates more rapidly than the lower-molecular-weight compound. In order to determine the relationship between permeability constant and molecular weight, the flux was measured for each alcohol in the series as it penetrated from a saline solution.

Average permeability constants (k_p), computed from these data by Equation 1, are shown in Fig. 4. According to these data, the permeability constant increases throughout this series as the molecular weight increases. The larger octanol molecules penetrate approximately 100 times faster than do the smaller methanol molecules.

3. *Vehicle*

The flux of polar and nonpolar alcohols was measured as they penetrated from polar and nonpolar vehicles. In most of these experiments, a 0.2 M solution was used. Average permea-

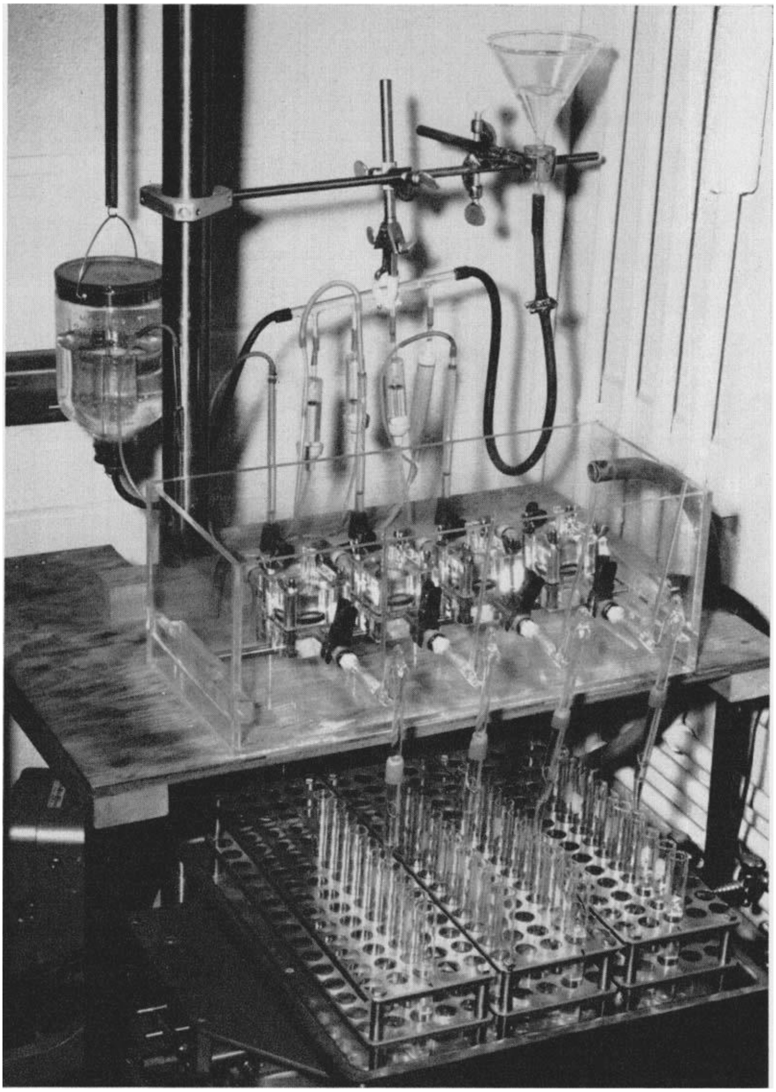

FIG. 2. Chamber assembly

bility constants, computed from the data obtained, are shown in Table I. It is seen that the polar alcohols, ethanol and propanol, penetrate more rapidly from nonpolar vehicles (isopropyl palmitate, olive oil and mineral oil) than from the polar vehicle, saline. The nonpolar alcohols, pentanol and octanol, penetrate more rapidly from saline. It is not yet clear why both polar and nonpolar alcohols can penetrate in only trace amounts from the polar vehicle, polyethylene glycol 600.

With nonpolar vehicles, steady state is not always attained within the first 24 hours. Many of the permeability constants given in Table I apply to penetration that occurred during the interval between 24 and 48 hours.

4. *Concentration of Pentanol in Olive Oil*

Pentanol and olive oil are miscible in all proportions; a 5 M solution is a mixture of the two materials in approximately equal volumes. Fig. 5 shows the flux of pentanol from olive oil solutions of varying concentration up to 5 M. Flux is nearly a straight-line function of concentration for weak solutions. As the concentration is increased above 1 M, however, flux does not continue to rise, but levels off and finally decreases as the concentration is further in-

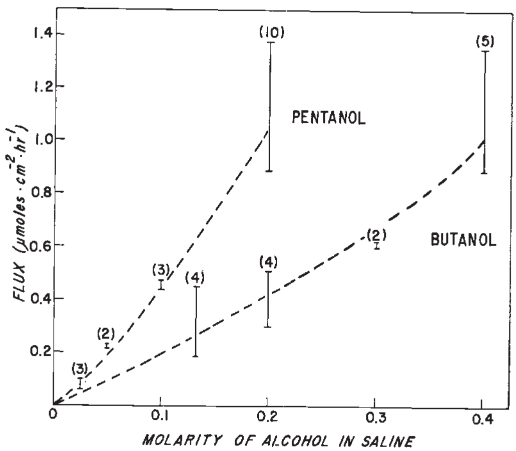

FIG. 3. Penetration of butanol and pentanol from saline solutions. Figures in parenthesis indicate number of experiments at the given concentrations.

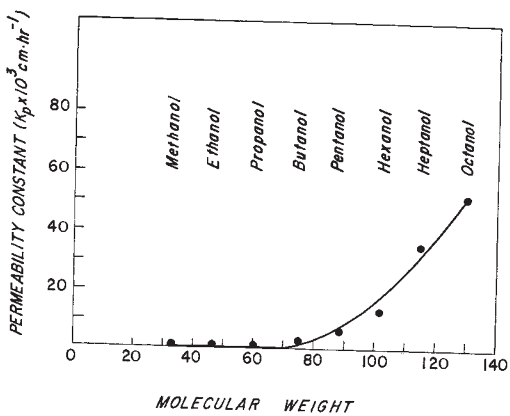

FIG. 4. Permeability constants of various primary alcohols when penetrating from saline solution.

TABLE I

Permeability constants ($k_p \times 10^3$ cm·hr^{-1}) for various alcohols when penetrating from different vehicles

Alcohol	Vehicle				
	Saline	Polyethylene glycol 600	Isopropyl palmitate	Olive oil	Mineral oil
Ethanol	0.3	<0.1	12.0	3.0	2.5
Propanol	1.0	<0.1	6.7	4.0	4.5
Pentanol	6.0	<0.1	1.0	1.2	1.5
Octanol	52.0	<0.1	0.1	<0.1	<0.1

creased. There is even less penetration from pure pentanol (not shown in Fig. 5) than from a 5 M solution in olive oil.

Data presented in Fig. 5 are permeability constants at about 24 hours. Although steady state may not have been reached at 24 hours for solutions between 1.5 and 4.0 M, a curve of steady-state rates will have the same general shape as that in Fig. 5.

DISCUSSION

Within the limits of these experiments, it has been possible to show that flux increased as the concentration of alcohol in saline rose. At low concentrations, the deviation from Fick's Law was not great. In a physical-chemical analysis of percutaneous absorption, Higuchi (3) has shown that one may expect flux to be a straight-line function of concentration. Treherne (4) observed this to be the case when methanol penetrated excised rabbit skin from an aqueous solution. Cotty *et al.* (5) have shown that as the concentration of methyl salicylate in mineral oil increases there is an increase in its penetration through the intact skin of living rabbits. Although older data, obtained by less accurate methods, may have left some doubt that an increase in the concentration of a penetrant might cause an increase in its flux, the occurrence of such an increase under many conditions no longer seems to be in doubt.

In order to discuss both the relationship between permeability constants and molecular weight, and the observations concerning penetration of the alcohols from various vehicles, it is desirable to expand Equation 1. Inherent in the permeability constant (k_p) are the factors: (*a*) distribution coefficient of the penetrant between the membrane and the vehicle (K); (*b*) the over-all membrane diffusion constant (D), and (*c*) the thickness of the membrane (δ).

$$k_p = (KD/\delta) \quad (2)$$

In this study of the penetration of alcohols through skin, the stratum corneum is considered "the membrane."

The mechanism whereby alcohols penetrate the skin now seems to involve transfer of an alcohol from its vehicle to the stratum corneum and subsequent diffusion of that alcohol through

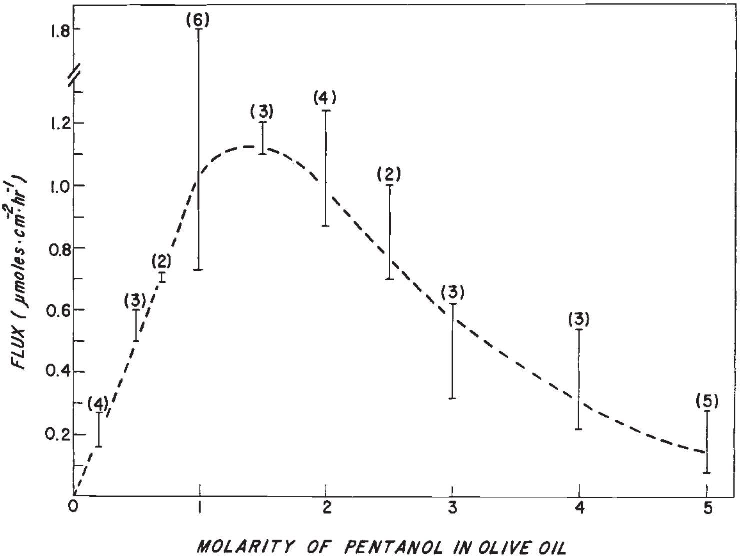

FIG. 5. Penetration of pentanol from olive oil solutions. Figures in parenthesis indicate number of experiments at the given concentrations.

the stratum corneum. This transfer is a function of the distribution coefficient of the alcohol between the stratum corneum and the vehicle (K) and is determined by the relative solubility of the alcohol in the stratum corneum and the vehicle. The over-all membrane diffusion (D) can be correlated to molecular weight but will change no more than two-fold within the range of molecular weights of these alcohols; the alcohol with the highest molecular weight will diffuse least rapidly.

The distribution coefficients of the alcohols between stratum corneum and water have not been determined, but investigations are now underway that may make their measurement possible. In general the distribution between stratum corneum and water may be expected to parallel the distribution between nonpolar liquids and water; in this homologous series, the higher the molecular weight the higher will be this distribution coefficient. The nonpolar-liquid/water distribution coefficient of octanol may be more than one hundred times that of methanol, depending upon which nonpolar liquid is chosen. The high permeability coefficient (k_p) of octanol as compared to that of methanol results, therefore, from the high distribution coefficient (K) of octanol. The small differences found in the rate of diffusion (D) of the two alcohols will play only a minor role in determining the difference between their permeability coefficients.

With a similar process of analysis it is possible to explain the changes in permeability constant associated with changes of the vehicle. For a polar alcohol like ethanol, the stratum corneum/water distribution coefficient will probably be lower than the stratum corneum/olive oil distribution coefficient because water has a greater affinity than olive oil for ethanol. The reverse is true for a nonpolar alcohol like pentanol; olive oil has a greater affinity than water for pentanol and therefore the stratum corneum/olive oil distribution coefficient of pentanol will be less than its stratum corneum/water distribution coefficient.

As the concentration of pentanol in olive oil reaches high values, the mixture has a greater affinity for pentanol than does olive oil alone. Therefore pentanol is released from the vehicle more slowly when the concentration of pentanol is high than when it is low, and the flux of pentanol through the skin decreases (Fig. 5).

Other investigators (Treherne (4) and Nogami and Hanano (6)) have shown that there is a relationship between the nonpolar-liquid/vehicle distribution constants and the permeability constants. Stolar, Rossi and Barr (7) theorized that "a distribution coefficient principle may be operative, *i.e.*, the greater the solubility of the drug in the vehicle as compared to the sebum, the less the absorption through intact skin," although they had not yet measured such distribution coefficients.

The data presented here support the hypothesis that alcohols penetrate the skin by being transferred from the vehicle to the stratum corneum, and that this transfer depends on the relative solubility of the alcohols in the stratum corneum and the vehicle. Other mechanisms of penetration that have been proposed, *e.g.*, (*a*) diffusion of a solute (penetrant) through vehicle-filled channels and (*b*) penetration of the vehicle which serves as a "carrier" for the solute, are not applicable to the alcohols.

SUMMARY AND CONCLUSIONS

The rate at which the alcohols penetrate skin from saline solutions of low concentration is approximately a straight-line function of the concentration of the alcohol in the vehicle. The rate of penetration of pentanol from a solution of olive oil does not continue to increase as the concentration of pentanol becomes high; the rate becomes maximal at a given point and thereafter decreases as the concentration continues to rise.

Solubility characteristics play an important part in determining the rate at which the alcohols penetrate skin. As the solubility of the alcohols in nonpolar liquids increases from methanol to octanol, the rate of penetration from an aqueous solution also increases. The alcohols penetrate more rapidly from vehicles in which they are less soluble.

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