Sarin Transport across Excised Human Skin I: Permeability and Adsorption Characteristics

DALE E. WURSTER, JAMES A. OSTRENGA *, and LLOYD E. MATHESON, Jr.[x]

Received February 5, 1979, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication May 21, 1979. *Present address: Pharm Chem, Palo Alto, CA 94301.

Abstract □ The transport rates and permeability coefficients for the transport of sarin in solution across both hydrous and anhydrous excised human skin were determined quantitatively. An activation energy was determined from study of the temperature influence on the transport of sarin in solution across anhydrous callus membranes. The transport of pure sarin and of sarin as a water-soluble and water-insoluble gel was studied also. The adsorption characteristics of sarin on powdered keratin were studied to determine their role in permeation. Both equilibrium and nonequilibrium measurements were made, which allowed calculation of the heat of adsorption, the heat of activation for adsorption, and the heat of activation for desorption. The results show that interaction between the membrane and penetrant plays a significant role in sarin transport across human skin.

Keyphrases □ Sarin—transport across excised human skin, hydrous, anhydrous, temperature influence, activation energy, adsorption and desorption, powdered keratin, permeability □ Cholinesterase inhibitors—sarin, transport across excised human skin, permeability, adsorption □ Transepidermal transport—sarin, excised human skin, permeability, adsorption

Diffusion of various penetrants through the stratum corneum has been studied for years; however, few investigators have considered the effects of adsorption and desorption of the penetrant by the membrane on transport.

The purpose of this study was to elucidate some factors involved in the diffusion of the nerve gas, sarin[1], across excised human skin membranes. The role of physical adsorption in transport also was studied for both equilibrium and nonequilibrium conditions.

THEORETICAL

For the permeability studies, it was assumed that a diffusion process occurs and that the entire exposed area of the membrane participates. For the solution phase studies, diffusion can be represented by:

$$\log (C_0 - 2C_2) = (-2k/2.303)t + \log C_0 \qquad \text{(Eq. 1)}$$

where C_0 is the initial solute concentration to which the barrier is exposed; C_2 is the concentration at time, t, on the opposite side of the barrier in the permeability cell (1); and k is the permeability coefficient. The permeability cell shown in Fig. 1*a* was used.

In studying the transport rate of pure sarin and of sarin in the gelled state, it was not experimentally practical to have the same volume of diffusing medium on each side of the barrier. Therefore, the permeability cells shown in Figs. 1*b* and 1*c* were used.

To examine the effect of sarin adsorption by keratin on diffusion, both adsorption and desorption were studied. It was assumed that the solvent and adsorbate do not compete for the same adsorption sites. Since adsorption may be considered to be a reversible equilibrium process:

$$K = \frac{k_a}{k_d} = \frac{C_{\text{powder}}}{C_{\text{bulk}}} \qquad \text{(Eq. 2)}$$

where K is the equilibrium constant; k_a and k_d are the specific rate constants for adsorption and desorption, respectively; C_{powder} is the milligrams adsorbed per milliliter; and C_{bulk} is the milligrams per milliliter remaining in the bulk solution after equilibration. At temperatures T_1 and T_2, for equal adsorbent weights and equal bulk solution volumes:

$$\frac{K_2}{K_1} = \frac{X_2}{X_1} \qquad \text{(Eq. 3)}$$

where K_1 and K_2 are the equilibrium constants, and X_1 and X_2 are the amounts adsorbed at temperatures T_1 and T_2. From the integrated form of the van't Hoff equation:

$$\log \frac{X_2}{X_1} = \frac{\Delta H^\circ}{2.303R}\left[\frac{(T_2 - T_1)}{(T_1 T_2)}\right] \qquad \text{(Eq. 4)}$$

and the difference in the amount of adsorbate adsorbed at two temperatures, it is possible to calculate the heat of adsorption for a relatively small temperature range. In addition:

$$k_a = Ae^{-H_a^\ddagger/RT} \qquad \text{(Eq. 5)}$$

$$k_d = Ae^{-H_d^\ddagger/RT} \qquad \text{(Eq. 6)}$$

where $H_a^\ddagger$ is the heat of activation for adsorption, $H_d^\ddagger$ is the heat of activation for desorption, A is the frequency factor, and the remaining terms are as defined earlier. Then:

$$K = \frac{k_a}{k_d} = \frac{e^{-(H_a^\ddagger - H_d^\ddagger)}}{RT} = e^{-\Delta H/RT} \qquad \text{(Eq. 7)}$$

Thus:

$$\Delta H_a = H_a^\ddagger - H_d^\ddagger \qquad \text{(Eq. 8)}$$

When desorption is first order and with the assumptions that, upon desorption, the adsorbate does not return to the adsorbent surface and

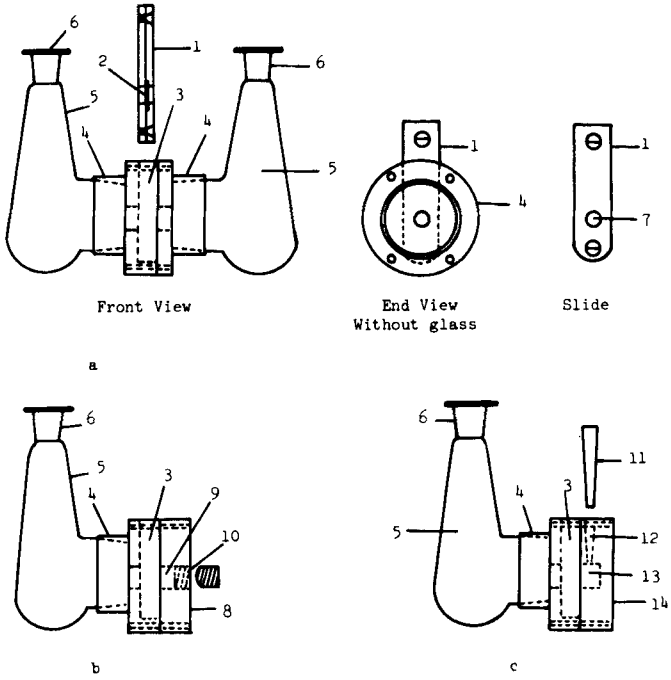

Figure 1—*Permeability cell for solution phase* (a), *for gel phase* (b), *and for pure penetrant* (c). *Key: 1, slide device; 2, membrane; 3, slide slot; 4, aluminum chassis; 5, glass chamber; 6, glass stopper; 7, slide orifice; 8, aluminum disk; 9, cavity for gel; 10, threaded port; 11, tapered pin; 12, tapered port; 13, cavity for pure penetrant; and 14, stainless steel disk.*

[1] Isopropoxymethylphosphoryl fluoridate.

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Table I—Average Transport Rates and Average Permeability Coefficients for Solution Phase Experiments

Experiment	Transport Rate, mg/cm²/hr	Permeability Coefficient, $k \times 10^4$/hr
Callus, anhydrous	0.05	2.81
Callus, hydrous	0.21	13.10
Stratum corneum conjunctum, anhydrous	0.21	19.45
Stratum corneum conjunctum, hydrous	0.49	28.77

the volume of desorbing solvent remains constant, it can be shown that (2):

$$\log \frac{X_e}{X_e - X} = \frac{k_d T}{2.303} \qquad \text{(Eq. 9)}$$

where X_e is the equilibrium amount of adsorbate in solution and X is the amount in solution at time t. The determination of k_d at various temperatures then permits calculation of $H_d^{\ddagger}$.

EXPERIMENTAL

Permeability Apparatus—The design of the permeability cell depended on whether the donor chamber contained a dilute solution, a concentrated gel, or pure sarin (Fig. 1).

Membrane Preparation—Callus membranes were sectioned from excised human callus to a 100-μm thickness. The stratum corneum conjunctum membranes were obtained by the well-known technique of stripping the human forearm[2]. The thickness of these membranes was 10–15 μm. Both the callus and the stratum corneum conjunctum membranes were stored first in ether followed by n-heptane, each for 24 hr. In the hydrous studies, the membranes were stored in a 100% relative humidity chamber for 24 hr prior to use.

The membranes were fixed in the slide of the permeability cell, and a small amount of silicone grease was employed to ensure a good seal. The areas exposed for the callus and the stratum corneum conjunctum membranes were 0.28 and 0.07 cm², respectively (6- and 3-mm orifices).

Gel Preparation—A water-insoluble sarin gel was prepared by making an 8% (w/v) solution of polymethyl methacrylate (mol. wt. 1.776 $\times 10^6$) in a 20-ml glass hypodermic syringe. The metal tip was threaded and sealed with a screw. The syringe was placed in a suitable container, sealed, immersed in a water bath at 25°, and shaken vigorously for 24 hr or until a uniform gel was produced.

A water-soluble gel was prepared similarly by making a 3% (w/v) solution of carboxypolymethylene[3].

Permeability Studies—For the solution phase studies, the permeability cell and slide were completely assembled. Then 50 ml of an n-heptane solution containing 7.8×10^{-3} M sarin, at the appropriate temperature, was placed in the donor chamber with the simultaneous addition of an equal volume of pure n-heptane in the receiver chamber. n-Heptane was dried by storage over anhydrous sodium sulfate. For the hydrous studies, the n-heptane was saturated with water at the appropriate temperature before the sarin solution in n-heptane was made. Water-saturated n-heptane also was used in the receiver chamber.

The permeability cells were maintained in a constant-temperature water bath with gentle shaking in the plane of the membrane. The transport rate was followed by withdrawing 1-ml samples from the receiver chamber at suitable times and analyzing for sarin. In the activation energy determination for the transport through anhydrous callus membranes, the rate was followed beginning with the lowest temperature (25°) and ending with the highest temperature (45°). The same set of membranes was used at all temperatures.

For the gel phase studies, 0.6 ml of the gel was placed on the membrane with a syringe through the threaded port on the side of the aluminum disk in cell-type 1*b*. The cavity was sealed with the set screw. Fifty milliliters of n-heptane was added immediately to the receiver chamber. An identical procedure for the pure penetrant studies was utilized, except that

Table II—Average Transport Rates of Sarin across Callus Tissue Membranes for Various Systems

Experimental Condition	Transport Rate, mg/cm²/hr
Sarin in n-heptane, anhydrous membrane	0.03
Pure sarin, anhydrous membrane	0.60
8% Polymethyl methacrylate gel, anhydrous membrane	0.49
3% Carboxypolymethylene gel, anhydrous membrane	0.55
8% Polymethyl methacrylate gel, hydrous membrane	1.1
3% Carboxypolymethylene gel, hydrous membrane	2.76

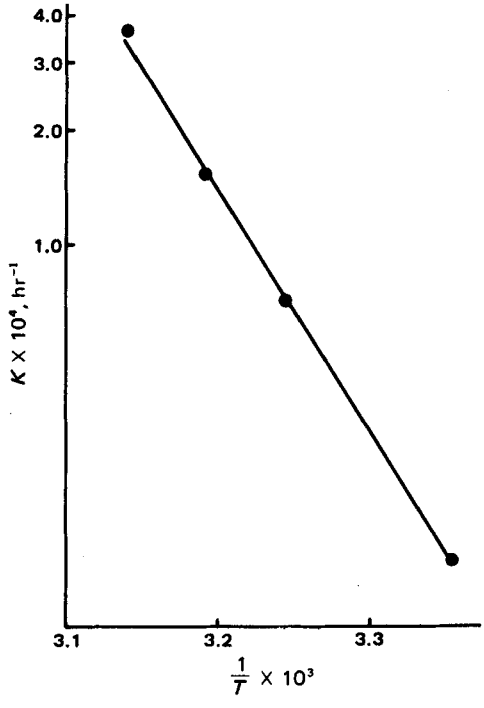

Figure 2—*Arrhenius plot for transport of sarin through callus membranes at 25, 35, 40, and 45°.*

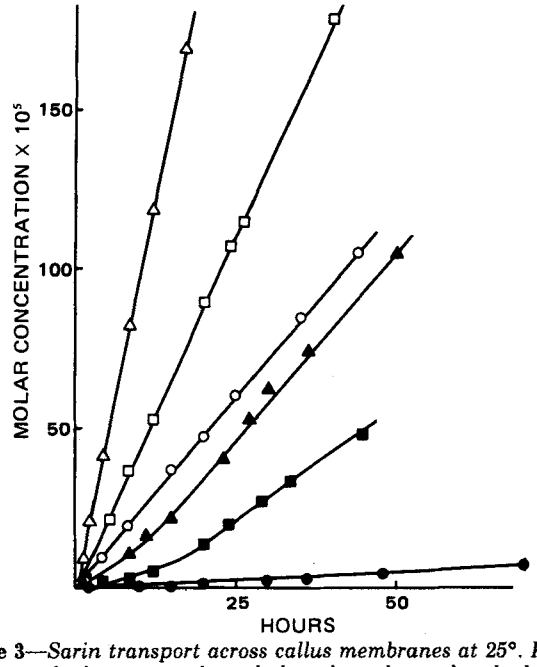

Figure 3—*Sarin transport across callus membranes at 25°. Key: ●, solution, anhydrous; □, polymethyl methacrylate gel, anhydrous; ▲, carboxypolymethylene gel, anhydrous; ○, pure anhydrous; □, polymethyl methacrylate gel, hydrous; and △, carboxypolymethylene gel, hydrous.*

[2] Scotch Brand No. 600 tape, 3M Corp., St. Paul, MN 55100.

[3] Carbopol 934, B. F. Goodrich Chemical Co., Cleveland, OH 44115.

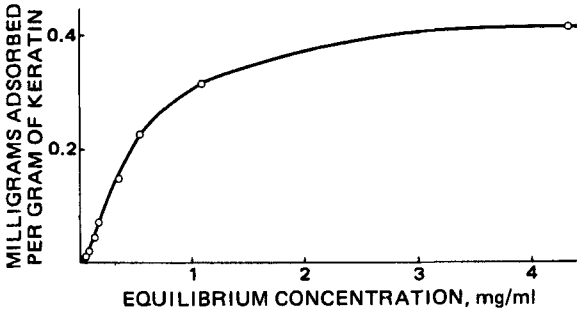

Figure 4—*Isotherm for sarin adsorption by powdered callus at 25°.*

sarin was injected from a syringe through the tapered part at the top of the aluminum disk in cell-type 1c. All gel and pure penetrant experiments were performed on the same set of membranes. Solution phase transport rates were then redetermined using a 7.74×10^{-3} *M* sarin solution in anhydrous *n*-heptane.

Assay—The analytical procedure (3) employed to determine the sarin concentration in *n*-heptane was as follows. One-milliliter samples were placed in a separator and the following solutions were added at the indicated time intervals: first, 2 ml of 0.625% (w/v) *o*-dianisidine in acetone; after 30 sec, 1 ml of 3% (w/v) hydrogen peroxide; after 1 min, 3 ml of 0.1 *M* tribasic sodium phosphate in redistilled water; and after 30 sec, 10 ml of xylene was added.

The mixture was shaken for 30 sec, and the xylene layer became yellow. After separation of the layers, the aqueous layer was removed, and the xylene layer was analyzed at 425 nm. The absorbance was directly proportional to the sarin concentration. The *o*-dianisidine was purified before use (4).

Adsorbent—Anhydrous excised callus tissue was pulverized in a blender[4] with the aid of dry ice. The dried powder that passed through a No. 170 standard sieve but was retained on a No. 200 sieve was used. The average particle size also was determined[5]. The powdered callus was extracted with anhydrous ether for 48 hr and then dried before use.

Adsorption Isotherm—A weighed quantity of the adsorbent was placed in a 50-ml volumetric flask, and 25 ml of a known sarin concentration in anhydrous *n*-heptane was added. The suspension was shaken in a constant-temperature bath at 25° for 3 hr. Under the conditions of these experiments, it was established previously that equilibrium was attained within this period. After equilibration, three 1-ml samples were withdrawn from the flask and filtered through glass wool in tubing[6] attached to the tip of the pipet.

The sarin concentration was determined by the analytical procedure

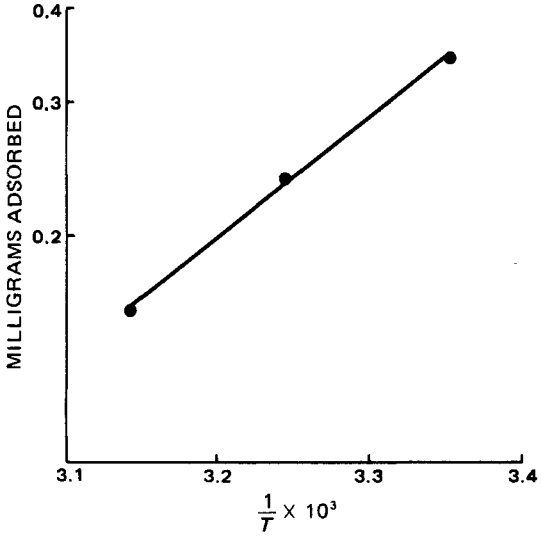

Figure 5—*Arrhenius plot of sarin adsorbed at 25, 35, and 45° by powdered callus. Initial sarin concentration was 44 mg/liter.*

[4] Waring Products Corp., Winsted, CT 06098.
[5] Subsieve sizer, Fisher Scientific Co., Pittsburgh, PA 15219.
[6] Tygon, Norton Plastics and Synthetics Division, Akron, OH 44309.

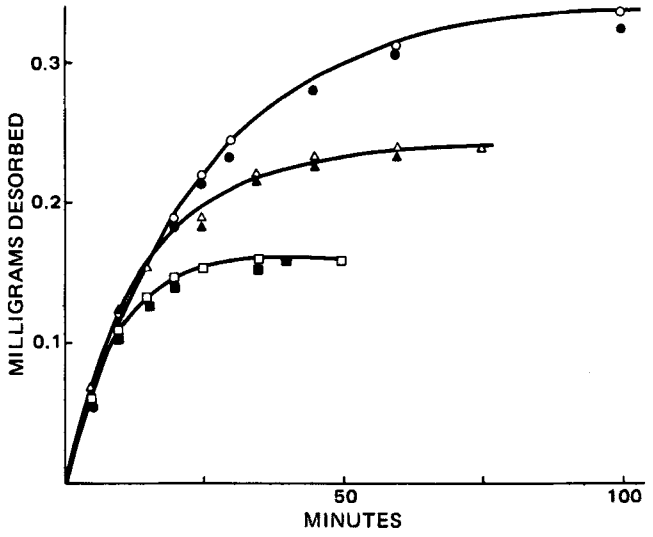

Figure 6—*Corrected desorption isotherms for sarin desorption from powdered callus at 25, 35, and 45°. Key: ○, ●, 25°; △, ▲, 35°; and □, ■, 45°.*

described earlier, and the average value for three samples was used in the calculations. The amount of sarin adsorbed per gram of powdered callus was calculated from the change in the bulk solution concentration. This procedure was repeated using weighed quantities of adsorbent and various concentrations of sarin in *n*-heptane.

Adsorption and Desorption Kinetics—To determine the heat of adsorption, equilibrium measurements of the amount of sarin adsorbed per gram of adsorbent were made at 25, 35, and 45°. After equilibration, the desorption rate was followed at each temperature. A known weight

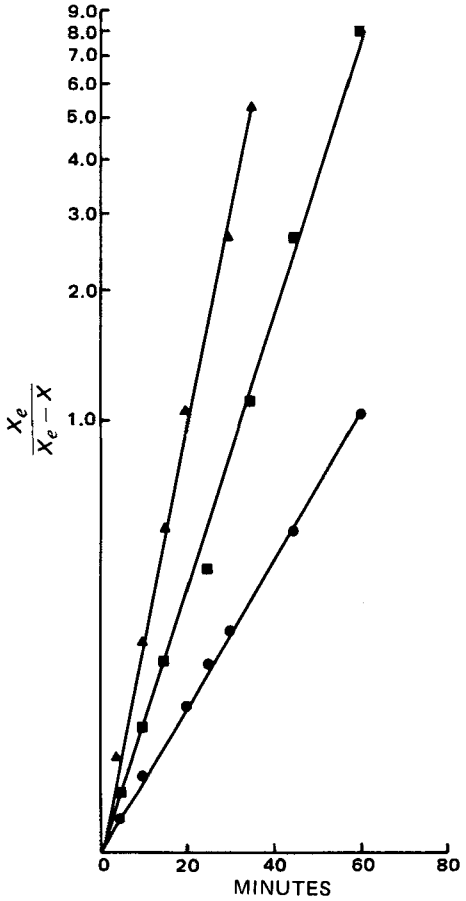

Figure 7—*First-order plots for sarin desorption from powdered callus at 25, 35, and 45°. Key: ●, 25°; ■, 35°; and ▲, 45°.*

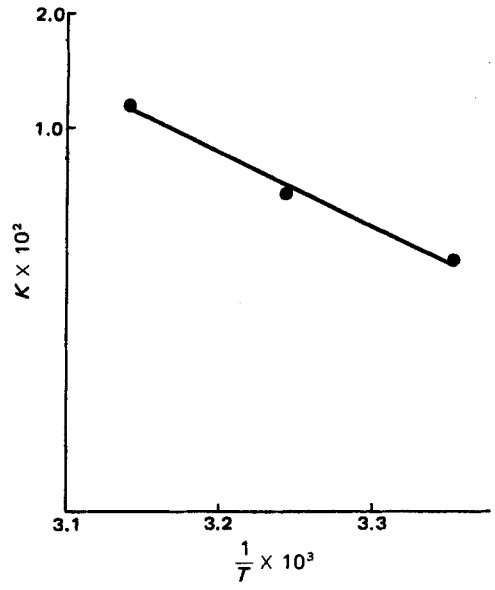

Figure 8—*Arrhenius plot for sarin desorption from powdered callus at 25, 35, and 45°.*

(~20 g) of ether-extracted powdered callus was placed in a 100-ml round-bottom glass-stoppered flask. Fifty milliliters of a known concentration (~44 mg/liter) of sarin in *n*-heptane was added. The flask was maintained at the appropriate temperature in a water bath and was shaken vigorously. Equilibrium measurements were made as described previously.

After adsorption was complete, the insoluble powdered callus was filtered out with a sintered-glass filter and washed on the filter with 50 ml of *n*-heptane using minimum vacuum. The powder was placed in 50 ml of *n*-heptane and maintained at the appropriate temperature during shaking. The desorption process was followed by withdrawing 1-ml samples periodically and assaying them for sarin. This procedure was repeated for each temperature.

RESULTS

Solution Phase Permeability—Average permeability constants and transport rates are shown in Table I. Each is the mean of 18–35 membranes for each solution system.

Activation Energy—Sarin transport through the same callus membranes was followed at temperatures of 25, 35, 40, and 45°. The activation energy calculated from the slope of the Arrhenius plot shown in Fig. 2 is 29.1 kcal/mol.

Gel Phase and Pure Sarin Permeability—The average sarin transport rates through callus membranes for each experimental series are given in Table II. Representative, but not averaged, plots of concentration *versus* time are shown in Fig. 3 on common coordinates.

Adsorption Studies—The isotherm for sarin adsorption by powdered callus at 25° is shown in Fig. 4. An Arrhenius-type plot of the sarin adsorbed at different temperatures from a solution with an initial sarin concentration of 44 mg/liter is given in Fig. 5. The heat of adsorption (ΔH_a) calculated from the slope was −7.2 kcal/mole. The average particle size at a porosity of 0.50 was 14 μm.

Desorption Studies—The corrected desorption isotherms are shown in Fig. 6. Data obtained from the desorption isotherm curves in Fig. 6 were utilized to obtain plots of log $[X_e/(X_e - X)]$ *versus* time (Fig. 7). The rate constants for desorption calculated from the slopes were 4.5×10^{-2} (25°), 6.75×10^{-2} (35°), and 11.5×10^{-2} (45°) min^{-1}. The heat of activation for desorption ($H_d^{\ddagger}$) calculated from the Arrhenius plot (Fig. 8) is 9.6 kcal/mole. Thus, according to Eq. 9, the heat of activation for adsorption is 2.4 kcal/mole.

DISCUSSION

Hydration increased the sarin transport rate in solution for both stratum corneum conjunctum and callus membranes. With the callus membranes, a four- to fivefold increase was found; with the stratum corneum conjunctum membranes, the rate increased ~1.5 times. The fact that hydration enhanced the transport rate agrees with previous studies (5–10). From Table I, it can be seen that the transport rate of sarin across stratum corneum conjunctum membranes was faster than that across callus membranes, and the difference in rate was in accordance with the difference in thickness of the two membranes. This result suggests that the two types of membranes have similar barrier properties.

Since sarin transport in solution followed the discussed mathematical models for diffusion, the results suggest that a diffusion process is largely responsible for sarin penetration through these membranes. This result, along with the high activation energy for transport, strongly supports the theory of transepidermal penetration. If the permeability mechanism were largely the transport of the penetrant *via* diffusional pores, a much lower value for the activation energy would be characteristic of such a process. The high value for the transport activation energy also suggests that the penetrant and the membrane may interact strongly.

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