Water Vapor Sorption and Desorption by Human Callus I: Anomalous Diffusion

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Abstract □ The rates of sorption and desorption of water in membranes of varying thickness of excised human callus were determined for several relative vapor pressures and temperatures. Under the conditions studied, the diffusion of water in these membranes was interpreted as non-Fickian in nature. It was concluded that at low water concentration this type of membrane exists in the glassy state. The effect of the relative vapor pressure on the water sorption rates was examined in the context of the free volume theory.

Keyphrases □ Diffusion—anomalous, water vapor sorption and desorption by human callus □ Sorption—water vapor, by human callus, anomalous diffusion □ Desorption—water vapor, by human callus, anomalous diffusion

The hydration of the skin has been found to increase the percutaneous absorption rates of several substances in humans (1). *In vivo* studies have demonstrated this effect for salicylate esters (2) and methyl ethyl ketone (3); whereas, an *in vitro* study has shown the same effect for sarin (4). The transport of water itself in skin is affected similarly (5–7). In an early study (8), water was shown to plasticize human callus when the concentration was ~10%. Since the glass transition temperature of stratum corneum is reported as 53° (9), it might be anticipated that non-Fickian diffusion of water in stratum corneum would occur below this temperature. The purpose of this study was to study the rates of water vapor sorption and desorption, to elucidate the nature of the diffusional process, and to provide a theoretical basis for the above observations.

THEORETICAL

The experimental system can be defined as consisting of water in the vapor state in equilibrium with sorbed water in a membrane of human callus. The rate of sorption to attain the equilibrium amount (Q_e), however, is considered to be controlled by the rate at which water molecules diffuse in the membrane. For isothermal diffusion in a two-component system, the rate of diffusion is governed by Fick's second law:

$$\partial C/\partial t = \partial/\partial x\,[D(C)(\partial C/\partial x)] \qquad \text{(Eq. 1)}$$

where C is the concentration of the diffusing molecules in the system at time t and distance x, and $D(C)$ is the mutual diffusion coefficient of the system, assuming that diffusion is one-dimensional and the diffusion coefficient depends on concentration only. The initial and boundary conditions assumed for sorption are:

$$C(x, 0) = 0, 0 < x < L \qquad \text{(Eq. 2)}$$

$$C(0, t) = C(L, t) = C_e, t > 0 \qquad \text{(Eq. 3)}$$

and for desorption:

$$C(x, 0) = C_e, 0 < x < L \qquad \text{(Eq. 4)}$$

$$C(0, t) = C(L, t) = 0, t > 0 \qquad \text{(Eq. 5)}$$

where L is the thickness of the membrane, and C_e is the equilibrium concentration at given temperature (T) and pressure (p). Solution of Eq. 1, subject to Eqs. 2–5, indicates (10) that the fractional uptake, $Q(t)/Q_e$, should theoretically increase linearly with $t^{1/2}$ during the initial period for both sorption and desorption, and the initial slope, $K(C_e)$, of the plot of $Q(t)/Q_e$ *versus* $t^{1/2}$ is given by (11):

$$K(C_e) = 4\bar{D}^{1/2}(C_e)/\pi^{1/2}L \qquad \text{(Eq. 6)}$$

In this instance, $Q(t)$ is the amount sorbed or desorbed at time t and $\bar{D}(C_e)$ is the mean value of the mutual diffusion coefficient over the concentration range taken. Thus, the slope will vary with C_e for any given membrane. It was also shown that for $D(C)$ increasing with C, $K(C_e)$ for sorption is always greater than $K(C_e)$ for desorption, the difference being an indication of how strongly $D(C)$ depends on C for the given concentration range. For D independent of C, it can be seen from Eq. 6 that for a given membrane, the slope K will be independent of C_e and identical for both sorption and desorption. It can also be seen that when $Q(t)/Q_e$ is plotted against $t^{1/2}/L$, the slope should be independent of L.

Deviations from these theoretical predictions for the conditions considered, however, have been observed in many polymer–diluent systems (12–14). These deviations generally implicate the role of slow relaxation processes for glassy polymers (14, 15).

To meet the conditions imposed on the mathematical representation of the system, thin membranes of callus devoid of lipid were used. Since kinetic data for the initial period were emphasized and studies were limited to the relative vapor pressures below unity, any swelling effect on the membrane thickness was considered negligible. Thus, from measurements of $Q(t)$ as a function of time for varied relative vapor pressures and thicknesses at constant temperatures, the nature of the diffusion process for the membrane–water system can be examined.

EXPERIMENTAL

Sorption–Desorption System—The system, constructed of glass[1], consists of a sorption tube connected to a manifold leading to both the vapor source and the vacuum system. A sensitive quartz spring[2] was placed inside the sorption tube. The entire sorption system was enclosed in a thermostated chamber and the temperature was controlled to within ±0.05°. A two-stage mercury-vapor diffusion pump[3] backed by a two-stage mechanical forepump[4] provided a base vacuum of $<10^{-4}$ torr. For measuring the extension of the spring, a precision cathetometer[5], which

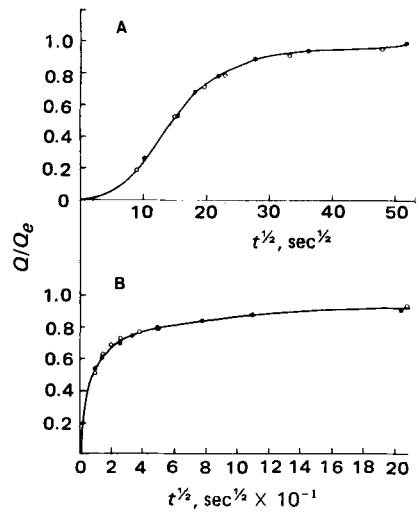

Figure 1—*Sorption and desorption of water vapor in human callus (Membrane 3) at 28°, p/p° = 0.84. Two repeated experiments. Key: (A) sorption, (○) Spring A; (●) Spring B; (B) desorption, (○) Spring A; (●) Spring B.*

[1] Pyrex.
[2] Worden.
[3] Pope, model 20045.
[4] Welch, model 1400.
[5] Gaertner, model M912.

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Table I—Relative Vapor Pressure ($p/p°$) of the Saturated Salt Solutions

Salt	$p/p°$ 20°	30°	40°
Sodium tartrate[a]	0.91	0.91	0.91
Potassium chloride[b]	0.86	0.84	0.83
Sodium chloride[b]	0.75	0.75	0.75
Cupric chloride[b]	0.68	0.67	0.67
Magnesium nitrate[b]	0.52	0.52	0.51
Magnesium chloride[b]	0.33	0.32	0.31

[a] Data from Ref. 17. [b] Data from Ref. 18.

Table II—Physical Constants of the Test Membranes

Membrane	Weight, mg	Diameter, mm	Thickness, μm	Density, g/ml, 25°
1	1.364 ± 0.005	4.85 ± 0.05	57.3 ± 1.6	1.288 ± 0.005
2	0.786 ± 0.005	4.585 ± 0.007	37.0 ± 0.5	
3	0.826 ± 0.005	5.032 ± 0.007	32.3 ± 0.4	
4	1.152 ± 0.005	4.941 ± 0.007	46.7 ± 0.5	

reads to 0.01 mm, was used. Thus, with a spring sensitivity of 20 mm/mg, a change in weight of 0.5 μg could be detected. This system was similar in principle to the one employed by Prager and Long (16).

The quartz springs were calibrated with small standard weights which were weighed to 0.1 μg on an electrobalance[6]. Four quartz springs (A, B, C, and D) having calibrated sensitivities of 9.36, 9.59, 18.73, and 17.60 mm/mg, respectively, were used.

Conventional salt solutions were employed to obtain the relative water vapor pressures in the system. The relative vapor pressures of these salt solutions (17, 18) are shown in Table I.

Membrane Preparation and Physical Measurements—Human callus was used as a convenient and readily available model for stratum corneum in an attempt to study water sorption, desorption, and the influences on the water transport mechanism in skin. Excised human callus tissue was microtomed to thin membranes of different thicknesses and delipidized in anhydrous ether as reported (4). To prepare a test membrane, the membrane was first partially hydrated and then cut with a 4.76-mm punch. The diameter of each test membrane was measured with a filar eyepiece micrometer[7] attached to an optical micrometer[8], which was calibrated with a micrometer disk[9]. The weights of the test membranes were obtained using an electrobalance[6]. All test membranes were dried over Drierite to constant weight. The thickness of the membrane was calculated from its diameter, weight, and density (19).

The weights, diameters, and calculated thicknesses of the membranes used in this study are given in Table II.

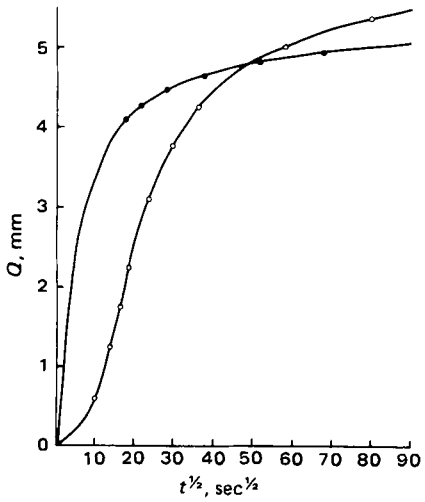

Figure 2—*Sorption and desorption of water vapor in human callus (Membrane 4) at 28°, p/p° = 0.91. Key: (○) sorption, (●) desorption, Spring B; Q_e (sorption) 5.58 mm.*

[6] Cahn, model 4200.
[7] Gaertner, model M202.
[8] Cenco.
[9] Bausch and Lomb.

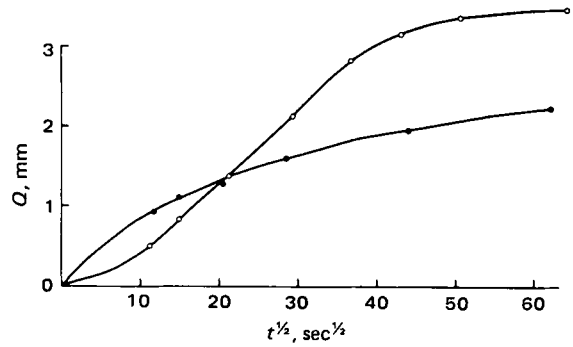

Figure 3—*Sorption and desorption of water vapor in human callus (Membrane 4) at 28°, p/p° = 0.67. Key: (○) sorption, (●) desorption, Spring C; Q_e (sorption) 3.53 mm.*

Sorption–Desorption Studies—For the sorption studies, the test membrane was attached to the quartz spring and kept at base vacuum at the given temperature until constant weight was reached. The membrane was then exposed to a given vapor pressure. The amount of water absorbed was followed by measuring the change in extension, $Q(t)$, of the spring with the cathetometer as a function of time until Q remained constant for a period of 24 hr. This value was taken as $Q_e(T,p)$. Upon the completion of the sorption run, the sorption tube was switched to the vacuum to initiate the desorption experiment. At that time $Q(t)$ represented the amount of water desorbed at the time t when Q was measured. Sorption and desorption rates were measured at relative vapor pressures of 0.32, 0.52, 0.67, 0.75, 0.84, and 0.91 and temperatures of 21, 28, 32, and 39°. The membrane thicknesses were 32.3, 37.0, 46.7, and 57.3 μm.

RESULTS AND DISCUSSION

Repeated experiments with the described system showed good reproducibility of the experimental procedure. A typical example showing the reproducibility of the experimental data is given in Fig. 1.

Effect of Relative Vapor Pressure—The rates of sorption and desorption were determined for various relative vapor pressures ($p/p°$) at 28° using Membrane 4. Paired sorption and desorption curves obtained for $p/p°$ = 0.91, 0.67, and 0.32 are shown in Figs. 2–4, respectively. The sorption curves obtained for four different relative vapor pressures are compared in Fig. 5. Similar results were obtained with test Membrane 1. Thus, the possibility of a constant, D, for the concentration range studied here does not seem plausible. Also, from the sigmoidal shape of the sorption curves and the relative positions of the sorption and desorption curves, it can be seen that the sorption and desorption of water vapor by the membranes are non-Fickian in nature. This would suggest that under the conditions studied, these membranes behave like glassy polymers, and some slow relaxation processes are taking place concurrently with the diffusion process when sorption occurs. The glass transition temperature of the callus was estimated to be ~55° (20). Thus, at 28° all experiments were started with a test membrane which was in a glassy state. At low water concentrations, for which the relaxation effect may be expected to be small, the rates of sorption or desorption could be essentially diffusion-controlled. This has been shown to be the case for a cellulose–water system (21), a wool–water system (22), and also appears to be true for the human callus–water system (Fig. 4). The sorption curve in Fig. 4, however, still exhibits the sigmoidal shape, which indicates that

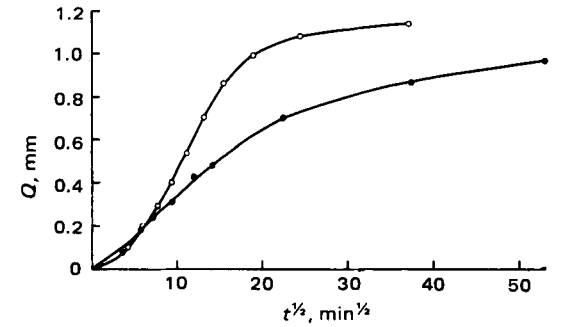

Figure 4—*Sorption and desorption of water vapor in human callus (Membrane 4) at 28°, p/p° = 0.32. Key: (○) sorption, (●) desorption, Spring C; Q_e (sorption) 1.14 mm.*

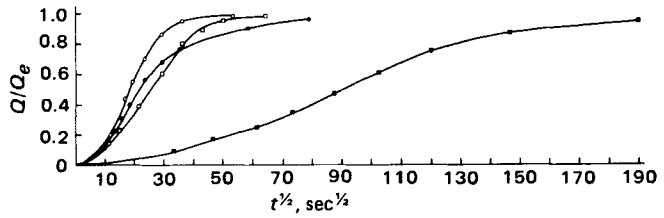

Figure 5—*Sorption of water vapor in human callus (Membrane 4) at 28°, p/p° = 0.32. (■); 0.67 (□); 0.84 (○); 0.91 (●).*

some relaxation effects are still present and cannot be totally ignored. For a cellulose–water system (21), this was previously attributed to a time-dependent surface concentration which approaches its equilibrium value shortly after the start of the experiment. A similar explanation could be suggested for the present system at $p/p° = 0.32$, which corresponds to an equilibrium water concentration of ~5% (w/w). It has been shown (12, 23, 24) that sigmoidal sorption curves can be obtained from the solution of Eq. 1 subject to the boundary condition of a variable surface concentration, $C_s(t)$. Both the theoretical consideration (25) and experimental evidence (23) for $C_s(t)$ have been utilized by others.

At high water concentrations, relaxation effects became more obvious (Figs. 2 and 3) and are not likely to be limited only to the surface region. A further complication may also arise. Since the glass transition temperature of the system is a function of the diluent concentration (26), these membranes may undergo transition from the glassy state to a rubbery state as sorption proceeds at the higher relative vapor pressures. Following Frisch (27), the glass transition temperature of the callus containing x (g/g) water can be estimated from the equation (28) based on the free volume theory:

$$T_g = T_g^\circ - (\beta/\alpha)x \qquad \text{(Eq. 7)}$$

where T_g is the glass transition temperature of the system, T_g° is the glass transition temperature of the callus, x is the water concentration (g/g), α is the difference between the thermal expansion coefficient above and below the glass transition temperature, and β is a parameter representing the contribution of water to the increase in free volume of the system. The value of β was found to be 0.16 for both collagen–water and wool–water systems (29). It also was shown (28) that for a limited range, β is independent of C and T. Using this same value for the present system and the suggested value of $\alpha = 4.8 \times 10^{-4}$ (deg^{-1}) for polymeric systems (30), T_g was calculated to be ~38° for $x = 0.05$ ($p/p° = 0.32$), and 15° for $x = 0.12$ ($p/p° = 0.60$). At $p/p° = 0.67$, which corresponds to an equilibrium water concentration of 0.16 g/g, the lowering of T_g will be even greater. Thus, at 28° and $p/p° = 0.67$, the callus membrane, initially in a glassy state, becomes rubbery whenever the concentration reaches its equilibrium value or lower, as long as it is sufficient to lower the T_g to <28°. This is in contrast to the absorption at 28° and the low $p/p° = 0.32$, where the membrane will remain in essentially the glassy state throughout the entire experiment.

While the exact shapes of the sorption curves in Fig. 5 reflect the total effect of both the relaxation and diffusion processes, in general they are in agreement with the presented analysis. In Fig. 5 the curves for $p/p°$ = 0.32, 0.67, and 0.84 all have an extended portion centered around $Q/Q_e = 0.5$, which is linear within the experimental error. The slopes of these linear portions are in order in accordance with an apparent diffusion coefficient increasing monotonically with the concentration. The slope of the curve for $p/p° = 0.91$, however, is smaller than that of the curve for $p/p° = 0.84$, indicating that the apparent diffusion coefficient decreases as the concentration increases above that corresponding to $p/p°$ = 0.84. A similar trend was confirmed with Membrane 1. The existence

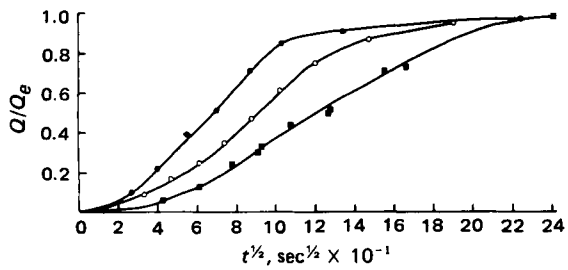

Figure 6—*Sorption of water vapor in human callus (Membrane 4) at p/p° = 0.32 and T = 21°. (■); 28° (○); 39° (●).*

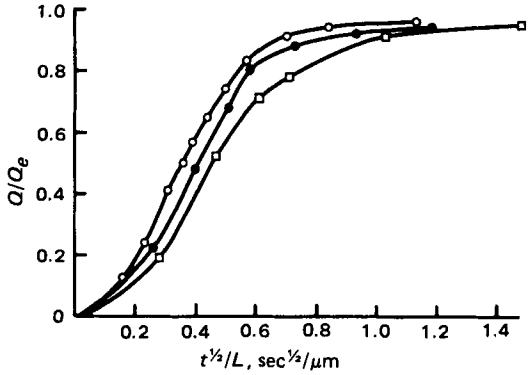

Figure 7—*Sorption of water vapor in human callus. Key: Membranes 1 (○) 57.3 μm; 2 (●) 37.0 μm; 3 (□) 32.4 μm at 28°; p/p° = 0.84.*

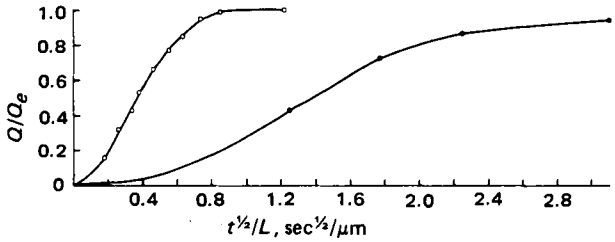

Figure 8—*Sorption of water vapor in human callus; Membrane 1. (○) 57.3 μm; and a human stratum corneum membrane (●) 9.7 μm at 28° and p/p° = 0.75.*

of a limiting value of the diffusion coefficient was also noted in a wool–water system (31). Thus, as absorption proceeds at 28° and $p/p° = 0.91$, not only is there a transition from glassy state to rubbery state for the callus–water system, but also a change in the functional dependence of D on C. This can be seen by the shapes of the curves for $p/p° = 0.84$ and 0.91 in Fig. 5.

The observation made by Blank (8) that the callus tissue became soft and pliable at 23° when it contained 10% water now can be understood. At this concentration, T_g of the system is effectively lowered to ~22°. Also, the same amount of water will not be sufficient to soften or plasticize the tissue at lower temperatures. The general effect of hydration on percutaneous absorption rates can be understood in the same context. The diffusion coefficients of organic vapors in glassy polymers are $\leq 10^{-10}$ cm^2/sec, while in the rubbery polymers they are in the order of 10^{-6} cm^2/sec (27).

Effect of Temperature—Absorption data obtained at 21, 28, and 39° for $p/p° = 0.32$ are compared in Fig. 6. The slopes of the linear portions of the curves increase as the temperature increases, which indicates that the apparent diffusion coefficient increases with the temperature, as would be expected. Since the relaxation time will also decrease as the temperature increases, the general effect of increasing the temperature to 39° was to reduce the role of the relaxation processes. The shape of the upper curve in the figure reflects this effect. A similar trend was seen for $p/p° = 0.67$ at these temperatures.

Effect of Thickness—In an attempt to confirm the non-Fickian mechanism, membranes of different thicknesses were used for the measurements of the rates of absorption under otherwise identical conditions. Results obtained at 28° and $p/p° = 0.84$ with Membranes 1–3 are compared in Fig. 7 in plots of Q/Q_e *versus* $t^{1/2}/L$. For a Fickian mechanism, the curves as plotted should be superimposable, but they are not. Repeated experiments for $p/p° = 0.75$ gave similar results. Such behavior was noted in a wool–water system (31) and a methylene chloride–polystyrene system (32). In both cases they were attributed to the time-dependence of D and/or C_s. The trend shown in Fig. 7 indicates that the apparent diffusion coefficient of the system studied depends on the membrane thickness which is smaller with the thinner membrane. Similar results are shown in Fig. 8 where a membrane, 1, of human callus having a 57.3-μm thickness is compared with a stratum corneum membrane of 9.7 μm at 28° and $p/p° = 0.75$. In the present report it appears that little difference other than thickness exists between these two different membrane types; however, this requires experimental verification.

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Pharmacokinetics of Probenecid Following Oral Doses to Human Volunteers

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Abstract □ The pharmacokinetics of probenecid were examined following single 0.5-, 1.0-, and 2.0-g oral doses to healthy male volunteers. Doses were administered following overnight fast, according to a randomized design. Plasma levels of probenecid were determined by high-pressure liquid chromatography (HPLC), using sulfamethazine as the internal standard. Mean peak probenecid levels of 35.3, 69.6, and 148.6 μg/ml were obtained at 3–4 hr following the 0.5-, 1.0-, and 2.0-g doses, respectively. Probenecid levels from the 0.5- and 1.0-g doses declined in apparent monoexponential fashion, with mean elimination half-lives of 4.2 and 4.9 hr. Interpretation of the 2.0-g data by a kinetic model incorporating first-order elimination resulted in a plasma drug half-life of 8.5 hr. When first-order elimination was replaced by a Michaelis–Menten-type function, the mean value of the resulting V_m/K_m ratios was 0.20, equivalent to a plasma drug half-life [$0.693/(V_m/K_m)$] of 3.8 hr. Plasma probenecid curves from all three dosages were successfully fitted to the saturable elimination model using nonlinear regression and numerical integration routines. The results suggest that probenecid elimination may be saturable at therapeutic dose levels.

Keyphrases □ Probenecid—pharmacokinetics following oral doses to humans, high-pressure liquid chromatography, elimination □ Pharmacokinetics—probenecid following oral doses to humans, high-pressure liquid chromatography, elimination □ High-pressure liquid chromatography—probenecid following oral doses to humans, pharmacokinetics

Probenecid is used for the treatment of gout and gouty arthritis and also as an adjuvant in therapy to prolong the plasma levels of other compounds, particularly the β-lactam antibiotics (1).

Although it has been used clinically for several years, the pharmacokinetics of probenecid are not well documented. It is efficiently absorbed after oral doses (2–4) with peak plasma concentrations occurring at 1–5 hr. Probenecid is 83–95% bound to plasma proteins at concentrations of 20–176 μg/ml (3). It is cleared from the body predominantly by metabolism, which occurs mainly by side-chain oxidation and glucuronide conjugation (3, 5). Only 5–11% of orally dosed probenecid is excreted in unchanged form in urine (6, 7).

The rate at which probenecid is cleared from plasma was shown to be dose-dependent in dogs (8) and humans (3). In the latter study, the plasma half-life of probenecid increased from 3–8 hr following a 0.5-g iv dose, to 6–12 hr following a 2.0-g iv dose in three subjects.

Since probenecid is extensively metabolized, dose-dependent elimination may be due to saturation of one or more metabolic pathways. However, a subsequent study demonstrated an unchanged pattern of urinary metabolites from oral doses of 0.5, 1.0, and 2.0 g of probenecid (6).

In view of the uncertainty regarding the nature and possible dose-dependency of probenecid pharmacokinetics, this study was undertaken to examine plasma levels of probenecid following 0.5-, 1.0-, and 2.0-g oral doses to healthy male volunteers.

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