Center by Grant No. HL19127 awarded by the Heart, Lung and Blood Institute, at the Armed Forces Radiobiology Research Institute by Research Work Unit 7452-00056 and 7452-00084, and by Grant No. ER60039 awarded by the Department of Energy. We appreciate the technical assistance of Mr. Ralph Will at GWU and E. Barron, N. Fleming, M. Flynn, J. Josza, J. Warrenfeltz, and M. Corral of the AFRRI. Animal research at AFRRI was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

We would like to thank Dr. Carl Kaiser of Smith Kline and French Laboratories for 4-bromoatropine; Dr. J. W. Bastian of Armour Pharmaceutical Co. for carbetapentane; Dr. W. Bartsch of Boehringer Mannheim for carazolol; and Dr. D. J. Marshall of Ayerst for propranolol. We would also like to thank G. Rosen for preparing the manuscript.

Percutaneous Absorption of Alkanoic Acids I: A Study of Operational Conditions

ZVI LIRON and SASSON COHEN ˣ

Received December 27, 1982, from the *Department of Physiology and Pharmacology, Tel Aviv University, Sackler School of Medicine and Institute for Biological Research, Ness Ziona, Israel.* Accepted for publication October 11, 1983.

Abstract □ The rate of penetration of propionic and butyric acids through excised porcine skin was determined *in vitro* in specific apparatus allowing optimal control of operational conditions. In one technique, the rate was followed by continuous pH-stat titration of acid appearing in the perfusate, in another, by periodic monitoring of [^{14}C]propionic acid in the perfusate. With the assumption that Fick's equation applies to the process of penetration, it was found that the permeability coefficient, K_p, increases with increasing mass of neat penetrant applied per unit area to the donor side, increases with increasing concentration of penetrant in *n*-heptane as vehicle, increases with increasing temperature, E_a = 11.4 kcal/mol, and decreases with decreasing perfusion rate of the acceptor side when this rate is smaller than 60 mL/h.

Keyphrases □ Absorption, percutaneous—alkanoic acids *in vitro*, porcine skin, permeability coefficients, operational conditions □ Alkanoic acids—percutaneous absorption *in vitro*, porcine skin, permeability coefficients, operational conditions □ Permeability coefficients—alkanoic acids through porcine skin, *in vitro* percutaneous absorption, operational conditions

The process of transdermal drug absorption is a subject of current interest in the areas of pharmaceutics, applied pharmacology, and toxicology. For passage through the skin, the penetrating molecule must move first through the stratum corneum, then into the viable epidermis, the papillary dermis, and the capillary walls into the bloodstream. Scheuplein and Blank (1) analyzed the diffusional resistance of the different skin layers, and it was shown clearly that the stratum corneum is overwhelmingly dominant.

This and the passive nature of the stratum corneum enabled application of Fick's equation to the transport process across the skin. A passive system has two main characteristics: (*a*) a delay period following contact of the penetrant with the surface, during which time the membrane itself becomes charged with the penetrant and (*b*) flow across the bulk of the membrane barrier at a steady rate, which lasts as long as the penetrant remains in adequate supply on one side and is being removed from the other. The steady-state flux of penetration, J_s, is given by:

$$J_s = \frac{K_m D(C_I - C_{II})}{X} = K_p \Delta C_s \qquad \text{(Eq. 1)}$$

where $K_p = K_m D/X$. K_p is the permeability coefficient, D is the diffusion coefficient, K_m is the membrane–solution partition coefficient, and X is the thickness of the membrane. ΔC_s expresses the concentration difference under steady-state conditions between the two phases at either side of the membrane. The permeability coefficient, K_p, is physically equivalent to the solute permeability coefficient at zero-volume flow, ω, as shown by Kedem and Katchalsky (2); the relationship is given by $K_p = RT\omega$. It was shown by many workers (1) that Fick's law holds fairly well for skin whether the penetrating substance is a gas, an ion, or a nonelectrolyte.

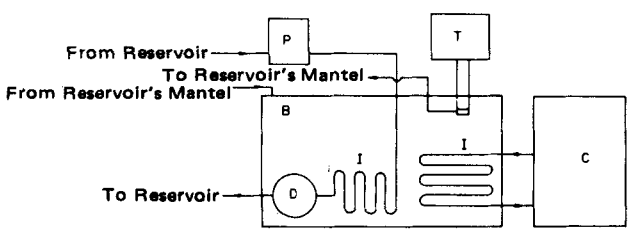

Figure 1—*Schematic representation of the pH-stat assembly. Key: (B) bath; (C) cooler; (D) diffusion cell; (I) immersion coil; (P) peristaltic pump; (T) thermostat.*

For optimal control of operational conditions, the penetration process is usually studied *in vitro*. Excised skin is used in diffusion cells in which a barrier of animal or human skin is interposed between two compartments, and the passage of compounds is measured from the epidermal surface on one side into a bathing fluid on the other side.

The present work is a pilot study of operational factors that affect the percutaneous absorption of propionic and butyric acids through excised porcine skin *in vitro*. Its main objective has been the generation of adequate knowledge to allow a systematic investigation of the permeation process by these and other members of this class of compounds. Concern with the alkanoic acids stems from an interest in the applicability of regular solution theory (3) to pharmaceutical sciences in general and to percutaneous drug application in particular. Recent accurate data (4) on the solubility parameter and molal volumes of a wide range of alkanoic acids suggested their use as candidates of choice in such a study, in preference to other potential permeating species, such as the series of alcohols used by Scheuplein (5). Compliance of the alkanoic acids with this theory is being presented in the second paper of this series (6).

The methodology used in this study is a modified extension of procedures that had been adequately described by earlier workers in this field, notably Ainsworth (7), Blank (8, 9), Wurster and Kramer (10), Scheuplein (5), Polano and Ponec

0022-3549/84/0400-0534$01.00/0

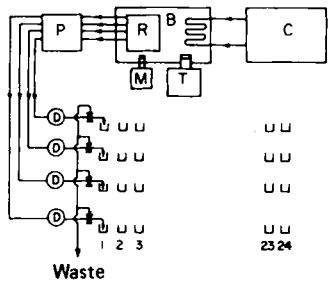

Figure 2—*Schematic representation of the automatic sampling skin penetration system, using radioisotope-labeled penetrant. Key: (B) bath; (C) cooler; (D) diffusion cell; (I) immersion coil; (M) centrifugal pump, (P) peristaltic pump; (R) perfusate reservoir; (T) thermostat; (⋈) T-valve, time actuated; (⊔) collecting vial.*

(11), and others (12). The main improvements over earlier procedures consisted in the design of the diffusion cells, which allowed much flexibility in the choice of experimental conditions, and in the automation of the perfusate sampling procedure, which was based on either of two independent methods: pH-stat titration of acid or the monitoring of isotope-labeled acid in the perfusate. Occasionally, both sampling procedures were used jointly, with remarkable agreement.

EXPERIMENTAL

Apparatus—Two procedures were used in the skin penetration studies. In one the penetration process could be followed directly by recording the titration of the acid appearing in the perfusate over a given period. This procedure however, was limited to relatively high concentrations of penetrants and to one diffusion cell at a time. In the automatic sampling system, using a radioisotope-labeled penetrant, one could use four diffusion cells in parallel and much lower concentrations of penetrant. The diffusion cells used in either procedure were almost identical, differing only in some external features.

pH-Stat System—This consisted of the diffusion cell assembly and the analyzing or titration assembly. The diffusion cell assembly is schematically shown in Fig. 1. The diffusion cell (D) was immersed in a thermostatic bath (B). The perfusate was made to flow in a closed circuit, being continuously sucked from the reservoir by a peristaltic pump (P), through an immersion coil (I), into the lower entrance of the diffusion cell, and through the exit of the diffusion cell, back to the reservoir. The flow rate in all these experiments

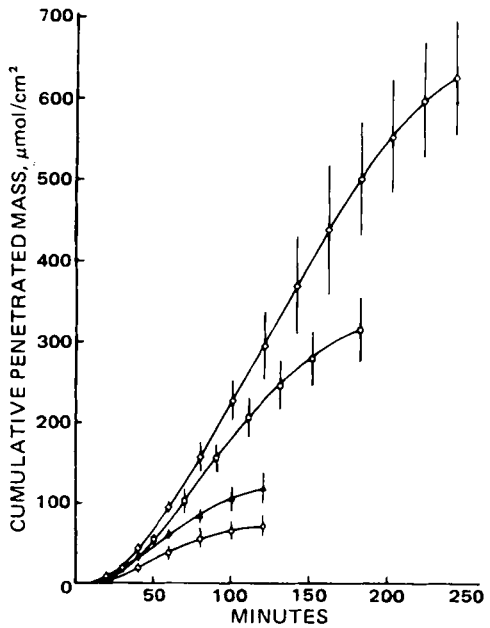

Figure 3—*Penetration of butyric acid through porcine skin at 37°C by the pH-stat procedure showing acid mass effect. Points and bars are means and* SE *from 3–5 runs. Key: (◇) 80 μL, 860 μmol; (□) 40 μL, 430 μmol; (△) 20 μL, 215 μmol; (○) 10 μL, 112 μmol.*

Table I—Penetration of Neat Butyric Acid Through Porcine Skin at 37°C [a]

Acid Applied, μL	Flux at Steady State (J_s), μmol/cm²/min	Permeability Coefficient ($K_p \times 10^3$), cm/min	Breakthrough Time, min
10	0.95	0.09	10
20	1.33	0.12	10
40	2.68	0.25	10
80	3.50	0.33	10

[a] Applied amount effect, determined by the titrimetric procedure.

was 1 mL/min. The temperature of the bath was controlled by a thermostat (T), equipped with a pump which was connected to the heating mantle of the reservoir. In cases where the room temperature was higher than the working temperature, a cooler (C) was used. The temperature in the bath was measured with a Brand thermometer, 0.1°C scale division.

The titration assembly was used in its pH-stat mode of operation. At that mode, the apparatus recorded the supply of the corresponding base required to keep the reservoir at a constant pH as a function of time. The equipment[1] included the following parts: recorder, pH meter, titrator, autoburet, stirring motor, and a reservoir equipped with a heating mantle and a combined microelectrode[2]. Nitrogen was bubbled into the reservoir to minimize access to atmospheric carbon dioxide.

The diffusion cell was of polytef and consisted of two parts assembled together to hold the skin sample. The lower part had a cylindrical hollow 1-cm² cross-circular area and 1-mL volume. Two stainless steel pipes of 1 mm i.d. entered the hollow, one at the bottom and one at the upper edge of the cylinder. Buffered solution flowing from the reservoir entered the diffusion cell through the lower pipe and left the cell, now with penetrant, through the upper pipe and back to the reservoir for titration. The skin to be tested was mounted on needle tips so that it constituted a tight barrier between the two compartments of the diffusion cell. The upper part of the diffusion cell contained suitable holes through which the needles passed, leading metal pins for convenience and accurate closing of the cell, and three screws for tightening the two parts of the cell to each other. The upper part was equipped also with a screw cap and a neoprene O-ring which enabled water-tight closure and immersion of the whole diffusion cell in the thermostatic bath.

Automatic Radioisotope Sampling System—A diagram of this system is shown in Fig. 2. The perfusate was contained in the reservoir (R) at a constant temperature maintained by the thermostat (T). When working at lower temperatures, the cooler (C) was used. The perfusate was sucked in parallel through four channels by the peristaltic pump and it was delivered into the lower entrance of each of the four diffusion cells. The perfusate containing the penetrant left each cell through the upper exit tube into the proper collecting vial. When working at high perfusate flow rates, the effluent from each diffusion cell was divided by a T-valve, electro-pneumatic actuated in a predetermined time cycle so that the proper amounts were in the vials and the rest was discarded. The 4-mL collecting vials were held in a special tray, in four rows of 24 vials each. The tray was shifted to each of its 24 positions, according to a predetermined time cycle. The tray shifting was activated by an electro-pneumatic system composed of a solenoid valve, a feeder, and two synchronic motors of 1 and 2 rph. When the tray reached its last position the whole system, including the peristaltic pump, was automatically switched off.

This experimental system was planned to work also at low perfusate flow rates and it became important to shorten, as much as possible, the distance from the exit of the diffusion cell to the sampling vial. This requirement could not be fulfilled when the cells had to be dipped into a thermostated bath. Therefore, it was decided to use diffusion cells of similar construction and design, but also equipped with a jacket for temperature control. To achieve good heat transfer, and yet work with noninteracting materials, the diffusion cells were made of stainless steel. In principle, each of the two parts of the diffusion cell could be temperature controlled independently, simulating the condition of the skin *in vivo*. In experiments in which the whole cell was kept at a single temperature, the jackets of the two parts of the diffusion cell were connected in series. The thermostatic fluid flowing through two diffusion cells was driven by the pump of the thermostat (T). The two other cells got their thermostatic fluid through the pump (M). The four diffusion cells were placed on a special tray, over and near the sampling vials tray.

Skin Samples—Intact porcine skin was used in all the experiments. Skin was clipped from the backs of male minipigs (Kibbutz Lahav strain) weighing

[1] Radiometer, Copenhagen.
[2] Ingold,type 2293.

Table II—Penetration of Propionic Acid (1 M *n*-Heptane Solution) Through Porcine Skin

Temperature[a], °C	Permeability Coefficient ($K_p \times 10^3$), cm/min	Breakthrough Time, min
50	6.72	4
37	3.00	8
30	1.64	10
25	1.25	20
15	0.81	40

[a] Temperature effect determined by titrimetric procedure.

~15 kg each. The skin was fastened to a hard board, enclosed in a plastic bag, and stored at ~−20°C until used. In preliminary tests, it was found that storage under these conditions for 2 months did not affect the penetration rate of propionic acid through the skin. The fat was carefully removed with a scalpel just before the skin sample was fastened in the diffusion cell. All samples of new skin were standardized before use by running a penetration experiment with 1 M propionic acid in *n*-heptane and by measuring the thickness of the stratum corneum, using the frozen-section technique (13).

Materials—All the compounds used in this study were of the highest analytical grade available commercially. The specific activity of [^{14}C]propionic acid was about 55 mCi/mmol; as a marker, it was diluted with "cold" propionic acid, to suitable working concentrations. The scintillation medium was prepared by mixing the following reagents, all scintillation grade: xylene (1 L), triton X-100 (0.5 L), 2,5-diphenyloxazole (6 g), and 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene (0.15 g). The perfusate medium was phosphate buffer, pH 7.4, composed of 140 mM NaCl, 0.4 mM KH_2PO_4, and 2 mM K_2HPO_4.

Operating Procedure—At the beginning of each experiment the titration assembly was calibrated with two buffer solutions, pH 7.0 and 4.0. A 25.0-mL volume of the corresponding phosphate buffer solution was introduced into the reservoir, and *via* the peristaltic pump the buffer solution flowed into the open diffusion cell and created a dome over the cylindrical hollow. The skin was mounted on the needles, pressed by the upper part of the diffusion cell, and fastened by screws into the proper position. In mounting the skin into the diffusion cell, care was taken to exclude air bubbles. The diffusion cell was then closed by its screw cap, dipped into the thermostatic bath, and preconditioned for 2 h to wash away the intrinsic acidity of the skin. This point, when reached, could be inspected easily from the trace of the recorder. In general, during the last half-hour of the preconditioning time no acid entered the perfusate.

After the preconditioning period, the diffusion cell was taken out of the bath, the cap was removed, and the proper amount of penetrant was applied to the skin. In all experiments except those in which the influence of the neat acid was studied, the penetrating medium was applied in a volume of 0.3 mL. The cap was replaced and the diffusion cell was returned to the thermostatic bath. As the penetrating acid entered the titration reservoir, it was automatically measured by titration with base. This allowed for direct recording of the penetration curve. In experiments where titration and radioactive analysis were simultaneously performed as a cross-check, 100-μL samples were taken from the reservoir at half-hour intervals. A 3-mL volume of scintillation medium was added, and the samples were counted in a scintillation counter. Triplicate runs were made for a given set of penetration conditions.

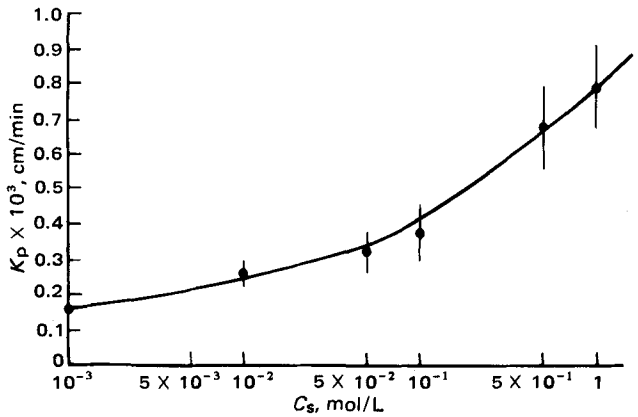

Figure 4—*Penetration of propionic acid from* n*-heptane solution through porcine skin at 25°C and at a perfusate flow rate of 3 mL/h by the radioisotope and pH-stat procedures, showing concentration effect. Points and bars are mean and* SE *from 3–5 runs.*

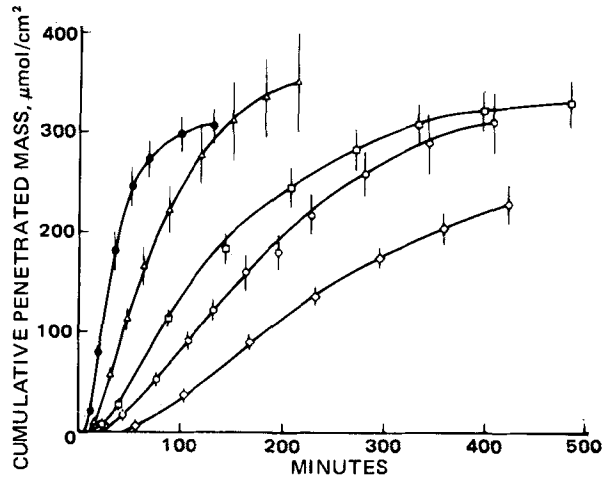

Figure 5—*Penetration of propionic acid (1 M* n*-heptane solution) through porcine skin by the pH-stat procedure, showing temperature effect. Points and bars are mean and* SE *from 3–5 runs. Key: (◇) 15°C; (○) 25°C; (□) 30°C; (△) 37°C; (●) 50°C.*

In the automatic sampling system the main procedure was essentially similar. The four diffusion cells were connected to the perfusate reservoir and the thermostatic fluid *via* flexible tygon tubing. The skin samples were mounted into the diffusion cell, the system was equilibrated for 2 h, and the penetrant was added to each of the diffusion cells. At the end of the penetration process the sampling vials were removed, and their contents were mixed with scintillation medium and counted. A 10-mL aliquot of scintillation liquid was added to 3 mL of sample; these proportions were found to give reproducible counting efficiencies. The first two samples were used as blanks for background counting immediately after the preconditioning period and before application of the penetrant.

The two techniques gave almost identical penetration curves and were complementary to each other. The titration assembly offered the advantage of direct recording of a continuous breakthrough curve and the convenience of following the penetration process in "real time." The system was simple to operate, but required use of relatively high donor phase concentrations.

In the automatic sampling system, use of radiolabeled penetrant increased the sensitivity of detection and estimation, making possible operation at low concentrations of the penetrant in the donor medium. But in this case, penetration analysis was not a continuous process as in the titration method. Samples of the perfusate were taken at distinct time intervals for analysis, the cumulative penetrating mass was calculated, and the penetration curve was drawn accordingly.

RESULTS AND DISCUSSION

Applied Mass Effect—The influence of the applied mass of the neat acid on the penetration characteristics was tested with butyric acid at 37°C. Experiments were done with 10, 20, 40, and 80 μL applied to the upper part of the skin; the corresponding penetration curves are shown in Fig. 3. The rates of penetration at steady state (J_s) and the corresponding permeability coefficients (K_p) are given in Table I; the values of K_p were calculated from Eq. 1 setting ΔC_s equal to the reciprocal molal volume of the neat acid. Breakthrough time was defined as the period taken for the first detectable penetrating molecules to appear in the perfusate.

With penetrating agents that do not interact with the skin membrane, it would be reasonable to assume that as long as the exposed skin area in the diffusion cell was covered with penetrant, the amount applied would not change the penetration curve. However, if the penetration rate depended on the amount of penetrant, as was indeed observed, then it could be reasoned that there occurred an interaction between the stratum corneum and the penetrant. From Fig. 3 it can be clearly seen that, in the early phase of penetration, the breakthrough time was similar for all amounts of acid applied. But as the process of penetration advanced further, the rate of penetration appeared to increase with the amount applied to the donor side. It is conceivable that butyric acid eventually affected the integrity of the stratum corneum barrier, which became less resistant as a barrier to the flow of the penetrant. The present finding agrees with those of Allenby *et al.* (14) who measured skin impedance changes after applying neat acids. Changes in impedance were found for neat acids, but almost no change was found for formic acid aqueous solutions (<80%).

Further insight into the effect of neat acid may be sought from a careful

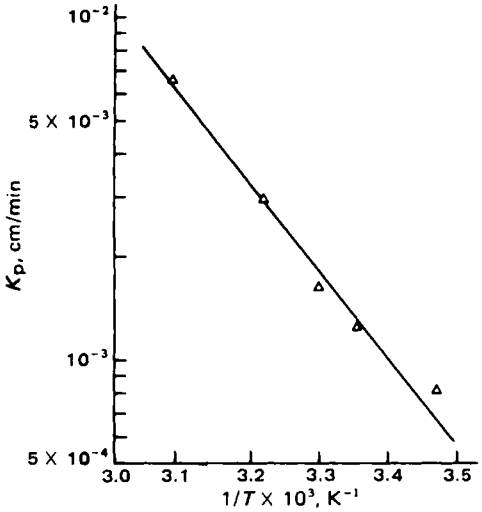

Figure 6—*Arrhenius plot of the dependence on temperature of the penetration of propionic acid (1 M n-heptane solution) through porcine skin: log* $K_p = 6.69 - 2.87 \times 10^3/T$ *(r = 0.99).*

consideration of the penetration curves (Fig. 3). There is first an exponential transient region, followed by a steady-state region, then an exponential decay region. The relative length of time for each region appears to depend on the amount of applied acid.

From the recovered fraction of the amount applied at the end of the steady-state region, it is conceivable that the eventual decline in penetration rate was due to the combined effect of depletion and a water back-diffusion process through which the acid on the donor side had become diluted to an extent that decreased its penetration. Such a process could also explain the apparent dependence of the penetration curves on the amount of compound applied, because it is expected to have a greater effect for smaller amounts of applied penetrant.

Concentration Effect—The effect of concentration was studied in the automatic sampling system using labeled propionic acid. The experiments were done at 25°C and at a perfusate flow rate of 3 mL/h; *n*-heptane solutions in the range of 0.001–1 M were used. Typical penetration curves were obtained, and the permeability coefficients, K_p, were calculated and plotted as a function of the penetrant concentration (Fig. 4). For convenience, the concentrations were drawn on a log scale. It can be seen that there is a smooth increase in the permeability coefficient as the concentration is increased. The increase in the permeability coefficient over a concentration range of three orders of magnitude is ~4.5.

The effect of concentration has been reported in many papers, but not much fundamental work has been done. It is generally taken as obvious that as the concentration increases, the flux also increases. Scheuplein and Blank (1) measured the penetration of butanol from aqueous solutions, in a concentration range of 0.01–0.3 M, and their results are consistant with the present findings; namely, there was a slight but continuous increase in the permeability coefficient as the penetrant concentration in the donor phase increased. The rate of change in K_p was larger for the higher concentrations. This change could be ascribed to altered barrier function as presumed to occur with large amounts of applied acid.

Temperature Effect—The effect of temperature on skin penetrations was studied for 1 M propionic acid in *n*-heptane. The experiments were carried out at 15°C, 25°C, 30°C, 37°C, and 50°C. The corresponding permeability coefficients are listed in Table II, and the penetration curves at these temperatures are shown in Fig. 5. A plot of log permeability coefficients against the absolute temperature reciprocal (Fig. 6) gave a straight line, the slope of which was used to derive the corresponding Arrhenius activation energy. The activation energy for propionic acid was found to be 11.4 kcal/mol. For comparison, Scheuplein and Blank (1) found values of 16.5 and 10 kcal/mol for the polar and nonpolar alcohols, respectively. It should be recalled that

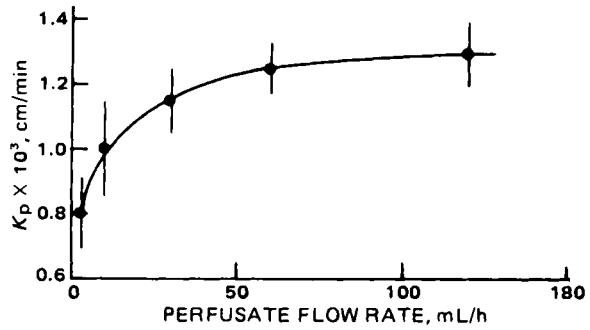

Figure 7—*Penetration of propionic acid (1 M n-heptane solutions) through porcine skin at 25°C by the pH-stat procedure, showing the effect of perfusion flow rate. Points and bars represent the mean and* SE *of 3–5 runs.*

they used a single membrane for the whole range of temperatures studied, in a procedure that took as long as 2 weeks to perform. In the present procedure, use was made of new skin samples for each experiment. The fit of results to a straight line (Fig. 6) is very good ($r = 0.99$).

This relationship, while useful, does not allow direct extrapolation to conditions that occur *in vivo*, where there is a temperature gradient across the skin. The sharp dependence of K_p on temperature implies a corresponding dependence of either K_m or D or both (Eq. 1) on the same variable. A temperature rise (or drop) across the skin may have a differential effect on each of the partitioning and diffusion steps in the overall permeation process.

Perfusate Flow Rate Effect—The perfusate flow rate is an operational factor which must be considered, especially if one intends to extrapolate from *in vitro* to *in vivo* conditions and also for a proper comparison of experimental data obtained from different sources. In a transport process where the diffusion through the membrane is the rate-limiting factor, it would be reasonable to assume that the penetration profile would not be affected by a change in the perfusate flow rate. But this would be true only for a certain range; the situation would change when the perfusate flow rate approaches zero, especially for the fast penetrants. In view of this, the effect of perfusate flow rate was considered in more detail. Accordingly, a study was made of the effect of the perfusate flow rate in the range of 3–120 mL/h, on the penetration rate of propionic acid (1 M in *n*-heptane). The results are shown in Fig. 7. As expected, the effect was pronounced at the lower perfusate flow rates, but at a flow rate of ~60 mL/h there was no further effect on permeability. Similar results were obtained by Tregear (15) for the penetration of tri-*n*-butyl phosphate through perfused porcine skin.

REFERENCES

(1) R. J. Scheuplein and I. H. Blank, *Physiol. Rev.*, **51**, 702 (1971).

(2) O. Kedem and A. Katchalsky, *J. Gen. Physiol.*, **45**, 143 (1961).

(3) J. H. Hildebrand, J. M. Pausnitz, and R. S. Scott, "Regular and Related Solutions," Van Nostrand-Reinhold, New York, N.Y., 1970.

(4) Z. Liron and S. Cohen, *J. Pharm. Sci.*, **72**, 499 (1983).

(5) R. J. Scheuplein, *J. Invest. Dermatol.*, **45**, 334 (1965).

(6) Z. Liron and S. Cohen, *J. Pharm. Sci.*, **73**, 538 (1984).

(7) M. Ainsworth, *J. Soc. Cosmet. Chem.*, **11**, 69 (1960).

(8) I. H. Blank, *J. Occup. Med.*, **2**, 6 (1960).

(9) I. H. Blank, *J. Invest. Dermatol.*, **43**, 415 (1964).

(10) D. E. Wurster and S. F. Kramer, *J. Pharm. Sci.*, **50**, 288 (1961).

(11) M. K. Polano and M. Ponec, *Arch. Dermatol.*, **112**, 675 (1976).

(12) W. Montagna, E. J. Van Scott, and R. B. Stoughton (Eds.), "Pharmacology and the Skin," Appleton-Century-Crofts, New York, N.Y., 1969.

(13) C. Blair, *Br. J. Dermatol.*, **80**, 430 (1968).

(14) A. C. Allenby, J. Fletcher, C. Schock, and T. F. S. Tees, *Br. J. Dermatol. Suppl.*, **4**, 81, 31 (1969).

(15) R. T. Tregear, "Physical Functions of the Skin," Academic, New York, N.Y., 1966.