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An investigation of in vitro percutaneous penetration enhancement of benzocaine by azone, dimethylsulfoxide, and 2-pyrrolidone

Amal Y. Benkorah
University of the Pacific

AN INVESTIGATION OF IN-VITRO PERCUTANEOUS PENETRATION ENHANCEMENT OF BENZOCAINE BY AZONE, DIMETHYLSULFOXIDE AND 2-PYRROLIDONE

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Amal Yousef Benkorah

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Amal Yousef Benkorah

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Department Chairman or Dean:

Thesis Committee:

Chairman

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ABSTRACT OF THESIS

This research utilizing full thickness human abdominal skin was designed to assess the *in vitro* percutaneous penetration of benzocaine by 1-dodecylazacycloheptan-2-one (Azone), dimethylsulfoxide (DMSO) and 2-pyrrolidone (2-P) under conditions of constant thermodynamic activity in the vehicle. The solubilities of benzocaine in Azone and 80/20, 60/40 and 40/60 V/V DMSO/water systems were found to be 254.17, 533.00, 68.60 and 2.51 mg/ml respectively. All three adjuvants demonstrated a significant but concentration-dependent enhancement of benzocaine penetration. On the basis of comparative analysis of the steady-state fluxes, Azone was most effective at the level of 5% V/V when drug concentration was twice the saturation solubility in the 20/80 PG/water gel. At higher Azone levels, any penetration enhancement effects were strongly negated by a corresponding decrease in skin/vehicle partitioning. Azone appeared to enhance penetration of benzocaine molecules by directly reducing the barrier function of the stratum

corneum. DMSO-induced enhancement of benzocaine penetration was observed over 40/80% V/V DMSO. Pretreatment studies strongly suggested that enhancement by DMSO is due to a significant but temporary effect on the epidermal barrier. The moderate enhancement of benzocaine penetration shown by 80% 2-P in water could be due to a decrease in diffusional resistance of stratum corneum brought on by a slow interaction between the stratum corneum and 2-P.

ACKNOWLEDGEMENT

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A warm appreciation is expressed to Mr. Rajesh Patel, my fellow graduate student, and to Mrs. Sandy McGowan for their kind help during the course of the research and preparation of the manuscript. A grateful acknowledgement is also extended to Dr. Albert Wong and Syntex Research for supplying the human cadaver skin samples. Finally, a very special gratefulness is expressed to my husband Mahmud, who has been a constant source of love and inspiration. Without his sacrifice, endurance and understanding, the author would not have been able to achieve her goals. To him and to the memory of my parents, I dedicate this thesis.

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INTRODUCTION

The process of transdermal drug absorption offers a great challenge to research workers in the fields of pharmacy and dermatology. The skin is one of the most impenetrable tissues of the body, for it functions as a barrier against invasion by microorganisms and viruses and simultaneously limits the escape of physiologically essential components, such as water, from the body. For passage through the skin, the penetrating molecule must move first through the stratum corneum, then into the viable epidermis, the papillary layers of the dermis, and the capillary walls into the blood stream. The viable tissue layers and the capillaries are relatively permeable and the peripheral circulation sufficiently rapid so that diffusion through the stratum corneum itself is rate limiting for the great majority of substances (1,2).

The stratum corneum consists of dense, highly compressed, partially desiccated cells. This layer has regions rich in protein separated by a lipoidal framework. The stratum corneum is formed and continuously replenished by slow migration of cells from the germinative basal layer of the epidermis. Studies have shown that this layer is constantly regenerated about every two weeks in the mature adult (3). An electron micrograph of a highly magnified

portion of the interior of a stratum corneum cell by Brady showed that this cell is filled with keratin filaments 60-80 A^{o} in diameter, distributed in an amorphous matrix of mainly lipid and non-fibrous protein. The conversion of viable epidermal cells into dried, compact, keratin-containing stratum corneum cells is the crucial event in the continuously developing epidermis that largely determines the low permeability of the skin. The filament-matrix ultrastructure of the intracellular keratin appears to play a role in the mechanism of diffusion, particularly in accounting for the selective permeability of polar and nonpolar molecules.

The mathematical expressions used to describe drug penetration through skin (4) are based upon the knowledge that passive diffusion is the predominant mechanism for penetration through human epidermis. Hence, Fick's law is generally applied to describe this process. Equation 1 describes membrane-limited transport under steady-state conditions:

$$J = \frac{D.P.\Delta C}{h} \qquad \text{(Eq. 1)}$$

The flux, J, represents the amount of drug passing through the membrane system per unit area per unit time, D is the diffusion coefficient within the membrane, ΔC is the difference in concentration across the membrane, h is membrane thickness and P is the membrane/vehicle partition coefficient.

The equation allows one to identify the factors that may be manipulated so as to control percutaneous absorption, namely the concentration and the partition coefficient of the penetrant. Although membrane thickness may remain constant at a given site, each penetrant may encounter a different type of barrier and the efficiency of the process of penetration may be subject to the physical-chemical properties of both penetrant molecules and those surrounding the passage (5,6,7).

Percutaneous absorption of local anesthetic agents has been the subject oa a number of reports (8,9,10). Benzocaine (ethyl aminobenzoate) is one of the most widely used local anesthetics in nonprescription products intended for relief of itch and pain. It has been reported (11) that local anesthetics in general are very poorly absorbed from the intact skin, but pass into the blood only if the skin is abraded or burned. This lack of efficacy of many nonprescription products in blocking the sensations of itch, burning and pain has also been supported by other in vivo studies of local anesthetics on intact and sunburned skin (12). Diffusion through the stratum corneum is the rate-limiting step in benzocaine transport and this accounts for the reported lack of efficacy of many nonprescription products.

The role of a vehicle in the transport of drugs into and through the skin is of fundamental importance for the

rational development of topical formulations. The physical-chemical characteristics of the components of the vehicle are a major consideration in the selection of the vehicle. Substances may be more rapidly released from vehicles having a low affinity for the penetrant. Vehicles with relatively low solvent power for incorporated compounds may induce more rapid penetration (13). In general, a compound must be at least partially soluble in its vehicle so that it can be readily released into the skin. High solubility may result in preferential retention of the drug in the vehicle. The physical properties of the components of the vehicle are also important in the degree of occlusion they provide leading to water retention in the stratum corneum layer.

A general approach towards affecting the topical bio-availability of drugs is to incorporate adjuvants which may either favor a high drug concentration in the stratum corneum or directly or indirectly affect the barrier function of the skin (14,15,16).

There are many formulations or substances which, when applied to the skin, can alter its suppleness and permeability properties which, in turn, depend to a large extent on the state of the stratum corneum. Of major interest are those substances which have a mild or even a reversible effect on the tissue. With these substances it may be possible to alter temporarily the properties of the skin

to promote effective drug delivery for both local and/or systemic effect.

Both ionic and nonionic surfactants have been investigated as adjuvants in dermatologic preparations for the purpose of enhancing the drug solubility and/or lowering the resistance to drug penetration without much success. For example, in a recent study of the effect of nonionic surfactants on benzocaine penetration through hairless mouse skin, Dalvi and Zatz (17) found that polyoxyethylene nonylphenol, a nonionic surfactant, produces a concentration-dependent increase in benzocaine solubility in water. The in vitro skin penetration flux, however, was found to be dependent only on the free benzocaine concentration in solution rather than total drug concentration in solution due to micellar entrapment.

Dimethylsulfoxide (DMSO), a polar, relatively nontoxic organic solvent, has been shown by many investigators to be an effective vehicle for increasing skin penetration (18,19, 20,21). Stoughton and Fritsch (18) have presented conclusive evidence to show that DMSO promotes the cutaneous penetration, in vivo, of the following substances: (a) a vasoconstrictor, naphazoline (Privine) hydrochloride; (b) an antiperspirant, hexopyrronium bromide; and (c) a corticosteroid, fluocinolone acetonide; and in vitro, hexopyrronium chloride and cortisol. It has also been shown that DMSO enhances the penetration of water. An approximately 20-fold

increase in the water transpiration rate occurs *in vivo* as compared with a 50-fold increase in water permeation through excised skin (2). DMSO lowers the skin's resistance to drug permeation but the specific mechanism is not fully understood. Kligman (2) has shown that the enhanced penetration offered by DMSO was largely reversible and little permanent damage to the stratum corneum occurred when it was removed and the tissue rehydrated. The application of DMSO to a biological membrane, such as the skin, effects a nonselective increase in tissue permeability which is not unique to DMSO but can be demonstrated, in varying degrees, by many other hypertonic solutions. In addition, there is a more specific property of DMSO which facilitates the movement of DMSO (and compounds which it has dissolved) across the skin and this appears to be the result of an increase in the size of passive flux channels through the membrane (19). This phenomenon may explain why compounds which are not well absorbed percutaneously from traditional vehicles may exhibit a dramatic increase in penetration when dissolved in DMSO (20).

Azone (1-dodecylazacycloheptan-2-one) has recently been shown to enhance the biologic activity of various topically active agents such as antibiotics and antifungals, glucocorticoids and antipsoriatic agents (22, 23). It is a colorless and relatively odorless compound that is non-irritating even when applied undiluted to the

human skin surface. The penetration-enhancing effect of Azone was observed with concentrations as low as 1%, which is much lower than that required with other enhancers, such as urea, DMSO and N,N-dimethylformamide. In addition, Azone caused practically no irritation to human skin even at concentrations of more than 50%. In light of these advantages, this substance has aroused considerable interest as a drug penetration enhancer for topical pharmaceutical formulations intended for both local and systemic effects.

Percutaneous penetration of both hydrophobic and hydrophilic molecules is enhanced with Azone, although more dramatic enhancements are usually seen with hydrophilic drugs. Stoughton (22) has shown that Azone significantly enhances the penetration of both anthracene, a hydrophobic compound, and 8-bromocyclic adenylic acid, a strongly hydrophilic compound. At low concentrations of anthracene, no penetration was observed without Azone and good penetration was observed with Azone. As the concentration of anthracene was raised, the effect of Azone was reduced until high concentrations of the drug showed little or no enhancement. Stoughton explained the foregoing result on the basis of the fact that a highly hydrophobic agent, when present in high concentrations, can penetrate the skin well enough so that it prevents Azone from acting to provide any additional effect. Examination of the effect of Azone

on 8-bromocyclic adenylic acid revealed that the steady-state rate of penetration of this compound through hairless mouse skin *in vitro* was enhanced 12-fold with 1% Azone relative to control. The precise mechanism is unknown, but it has been postulated that Azone alters the lipid and protein structures of the skin. Radioactive studies have shown that Azone is bound in the epidermis and corium and that relatively little is released from the skin.

Recently, 2-pyrrolidone has been investigated for potential skin penetration-enhancing properties by Barry and Southwell (24). They measured the steady-state fluxes *in vitro* for polar methanol, non-polar octanol, and an intermediate compound, caffeine, using human stratum corneum, and determined partition, permeability and the apparent diffusion coefficient. It was concluded that 2-pyrrolidone enhances permeation through the polar route of the skin by increasing the diffusivity, and reduces passage through the nonpolar route by decreasing diffusivity and partitioning. Stoughton (25) and coworkers combined 2-pyrrolidone with clindamycin in the treatment of acne vulgaris and suggested that the compound aided penetration of clindamycin into the comedo. In a study, five patients used clindamycin phosphate in 2-pyrrolidone for periods from 7 to 19 weeks. All five had complete suppression of growth of the acne organism in open comedones.

Low molecular weight polyols are used extensively in cosmetic and dermatologic practice. Propylene glycol in particular has been shown to increase the aqueous solubility of many drugs and also enhances their skin penetration when used as a cosolvent with water (26,27,28).

Recently, Lam (29) has examined a series of propylene glycol/water blends as potential vehicles for topical preparations of benzocaine. The role of Azone as percutaneous penetration enhancer for benzocaine solution in 60/40 propylene glycol/water was extensively investigated. The in vitro skin penetration of benzocaine from a series of dimethyl sulfoxide/water blends was also studied using human cadaver skin. These studies document penetration-enhancing effects of propylene glycol, dimethylsulfoxide and Azone primarily under conditions of decreasing thermodynamic activity. Could benzocaine penetration flux be further maximized by manipulating the concentration of drug, solvent or the penetration accelerator? Does the enhancement of benzocaine penetration by Azone involve a direct effect on the skin? What is the contribution of Azone as a solvent for benzocaine? How does it influence skin-vehicle partitioning? These are some of the questions that still need to be addressed.

Scope of the Present Study

This study was designed to help answer some of the aforementioned questions and further refine the formulation parameters for a topical preparation of benzocaine. *In vitro* skin penetration of benzocaine will be examined under conditions of constant thermodynamic activity with respect to penetrant benzocaine from 20/80 propylene glycol/water and dimethyl sulfoxide/water systems. The scope of Azone as a penetration accelerator will be further scrutinized to define its appropriate level of concentration in the vehicle and to shed more light on its mode of action as a penetration enhancer. In view of recent reports, the effect of 2-pyrrolidone on percutaneous penetration of benzocaine will also be examined.

EXPERIMENTAL

Materials

Benzocaine,[1] propylene glycol[2] (PG), dimethylsulfoxide[3] (DMSO), Azone,[4] 2-pyrrolidone[5] (2-P), klucel[6] (hydroxypropyl cellulose), saline,[7] absolute ethanol,[8] and double distilled water.[9] All chemicals were used as received from the manufacturers.

Solubility Studies

The solubility of benzocaine in pure Azone was determined at 25° ± 0.5°C by magnetically stirring a known excess amount of the drug in 5 ml Azone in a 10-ml glass-stoppered Erlenmeyer flask for a period of 48 hours. The stirring was stopped and an aliquot of the supernatant was filtered and appropriately diluted with ethanol. The study was done in duplicate and the concentration of benzocaine was determined spectrophotometrically at 252 nm. The

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1 "Baker" Grade, Lot. 847340, J. T. Baker Chemical Co., Phillipsburg NJ.
2 USP Grade, Lot. 909349, J. T. Baker Chemical Co., Phillipsburg NJ.
3 "Baker Analysed" Lot. 805340, J. T. Baker Chemical Co., Phillipsburg NJ.
4 Lot. 0157-132, Nelson Research, Irvine CA.
5 Lot. 713381, Fisher Scientific Comp., Fair Lawn NJ.
6 HF grade, Lot. 3027, Hercules Inc., Wilmington DE.
7 Sodium Chloride Injection USP, McGaw Lab., Irvine CA.
8 Ethyl Alcohol USP, D1-19425, Gold Shield Chemical Corp., Hayward CA.
9 Univ. of the Pacific, Stockton CA.

saturation solubility of the drug in Azone was obtained from the standard curve pretested for compliance with Beer's law.

The solubility of benzocaine in a series of DMSO/water mixtures (40 to 80% in 20% increments) was determined gravimetrically. An excess of benzocaine was weighed and added to 25 ml of DMSO/water mixtures in 50-ml glass-stoppered Erlenmeyer flasks containing a Teflon-coated magnetic stirring bar. The solution was stirred for a period of 48 hrs at $25^{\circ} \pm 0.5^{\circ}$. Drug was allowed to settle. The supernatant was filtered, and a known volume was placed in a preweighed 50-ml round bottom flask. The solvent was removed *in vacuo*[10] (rotatory evaporator) and the flask was reweighed to a constant weight to calculate the saturation solubility in each of these systems. Each determination was done in duplicate.

Preparation of Test Solutions, Suspensions and Gels

All solutions were prepared on a volume/volume basis unless indicated otherwise. Excess amounts of benzocaine were accurately weighed and carefully dispersed in the following media:

a. Propylene glycol in water (20 and 60% V/V)

b. Dimethylsulfoxide in water (40, 60, and 80% V/V)

c. 2-Pyrrolidone in water (10, 20, 40, and 80% V/V)

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[10] Rotavapor R-110/RE-120, Düchi Lab., W. Germany.

The dispersions of benzocaine in propylene glycol/water (20/80) were gelled with 4% (W/V) hydroxypropyl cellulose, after adding the proper concentration of Azone for the purpose of testing for the effect of this adjuvant as a skin penetration enhancer.

In Vitro Skin Penetration Studies

Skin Preparation: Full thickness human abdominal skin was obtained at autopsy and kept frozen until ready for use. Before the experiment, the skin was removed from the freezer and allowed to thaw gradually at room temperature. When the sample was pliable, it was placed epidermal side down on a dissecting board. All subcutaneous fat was then carefully removed with a scalpel. A section of skin derived from a single subject yielded skin pieces for 8-10 diffusion cells.

Skin Diffusion Cell: The cell consists of two parts (Fig. 1). The bottom part is a glass chamber with a sampling port and is enclosed by a water jacket which allows for circulation of water at selected temperature. A Teflon-coated magnetic stirring bar[11] was placed at the bottom of the chamber to ensure good mixing. The skin piece was placed in position on an O-ring between the two ball joints of the top and bottom chambers, providing a diffusion area of 2.01 sq. cm.

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[11] Spinbar, V.W.R., San Francisco CA.

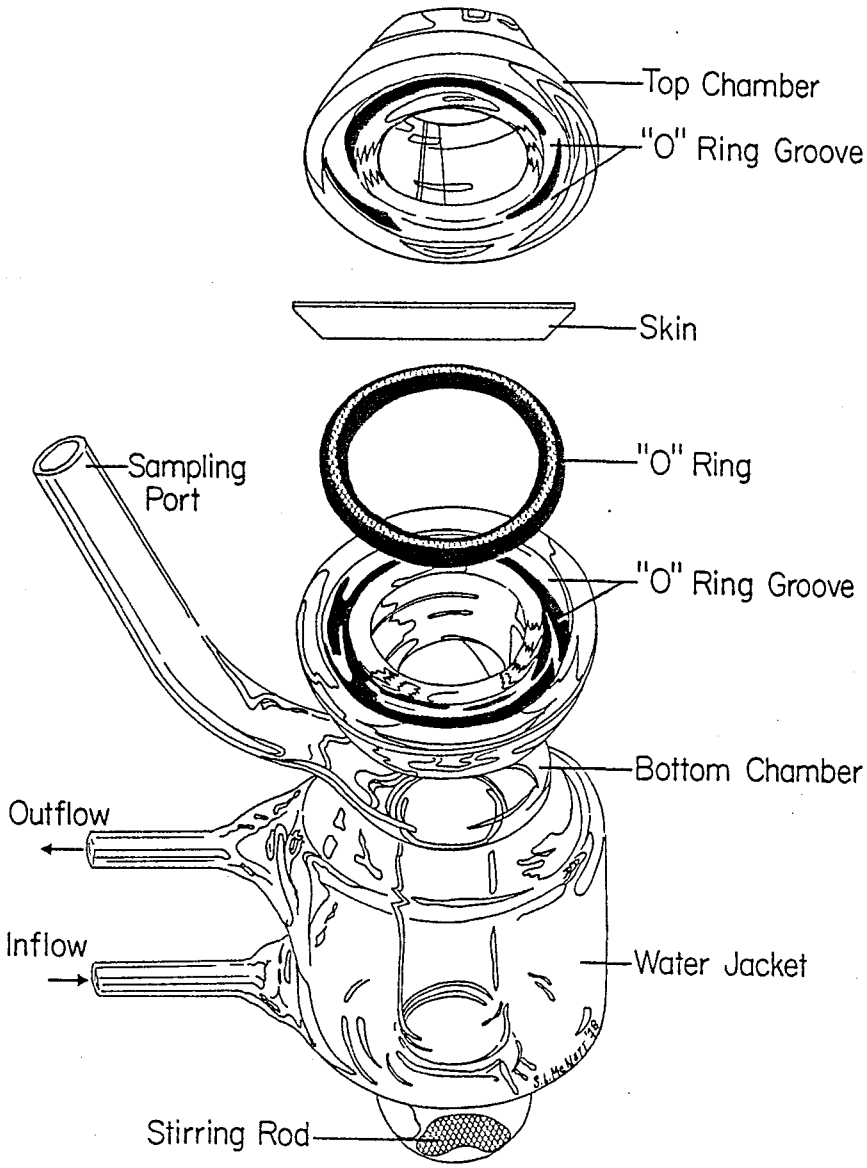

Figure 1. Diagrammatic representation of the diffusion cell used in penetration studies.

The top chamber was covered with parafilm and/or plastic wrap during all penetration studies to provide occlusion. Normal saline (6.2 ml) was injected into the lower chamber by means of a syringe and a piece of tubing.[12] Air bubbles that might be trapped on the dermal side of the skin were allowed to escape via the sampling port by carefully tilting the skin cell. Each cell was mounted on a magnetic stirrer. The temperature of the receptor fluid in the lower chamber was maintained at $37^{o} \pm 0.5^{o}$ by circulating water from a constant-temperature water bath fitted with a circulator pump.[13] The skin sample was allowed to equilibrate in this condition overnight. Before any formulation was applied to the skin, the saline in the bottom chamber was removed and replaced with fresh saline. Approximately 2 ml of the test formulation was applied to the skin surface in all cases.

All experiments were carried out for 12 hrs. Samples were withdrawn at appropriate time intervals and analyzed.

Pretreatment Studies: Unless otherwise indicated, all pretreatment studies involved surface application of the pure adjuvant on the excised human skin, mounted on the skin cell, for a predetermined period of time. Pieces were then rinsed with ethanol followed by water and wiped with a cotton swab to ensure complete removal of the excess

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[12] Norton Corp., Akron OH.
[13] Haake Model-FE, V.W.B., San Francisco CA.

adjuvant prior to conducting drug penetration experiment.

Pretreatment by soaking involved total immersion of the skin sample in the selected adjuvant for a predetermined period of time followed by the removal of excess adjuvant by successive rinsing with ethanol and water. The complete removal of excess adjuvant and water was ensured by wiping with a cotton swab as before.

Assay Procedure

Analyses for benzocaine in the receptor fluid were done on a spectrophotometer[14] fitted with a micro flow-through attachment. This allowed for small sample size and rapid sampling. The absorbance was recorded at 252 nm (29) and converted into concentration by means of a standard curve. Beer's law was obeyed throughout the concentration range studied (2.5 to 20.0 mcg/ml) (see Appendix A and B).

Data Treatment

Each release experiment was performed in duplicate and the average data were then used to draw the individual graphs plotting the amount penetrated per unit area against time. Regression analyses were performed on all of these plots to obtain penetration rates and other regression parameters followed by testing for paralellism and calculating the 95% confidence limits for each slope.

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[14] Spectronic 710, Bauch and Lomb, Rochester NY.

RESULTS

In these studies, in vitro penetration of benzocaine across human cadaver skin has been examined from a series of aqueous formulations primarily under conditions of maximum thermodynamic potential achieved by incorporating the drug in excess of its solubility in respective vehicles. The formulations included selected blends of Azone/PG/water, DMSO/water, and 2-P/water. The effects of Azone, DMSO, and 2-P upon percutaneous penetration of benzocaine were evaluated by either applying the appropriate formulation directly to the skin or by pretreating the skin with pure adjuvant. Since Azone is immiscible in aqueous systems, various Azone/PG/water blends were gelled with 4% hydroxypropyl cellulose to achieve uniform dispersion.

The following pages document the solubility and penetration data generated during the course of this investigation and also provide an analysis and interpretation of these results.

Solubility Studies

The solubilities of benzocaine in 40, 60, and 80 percent DMSO/water at $25^{\circ} \pm 0.5^{\circ}$ are shown in Table I.

The solubility of benzocaine in PG/water systems has been previously shown (29) to have a range of 2.00 mg/ml in water to 123.75 mg/ml in pure propylene glycol. The

TABLE I

Solubility of Benzocaine in DMSO/Water Mixtures at 25° ± 0.5°

DMSO/Water (% V/V)	Solubility (mg/ml)	Percentage Solubility
80/20	533.00	53.3
60/40	68.60	6.86
40/60	2.51	0.25

solubility of benzocaine in 20% and 60% propylene glycol in water (vehicles used during this study) was found to be 4.25 and 17 mg/ml respectively.

The solubility of benzocaine in Azone at $25^{o} \pm 0.5^{o}$ was found to be 254.17 mg/ml in sharp contrast to its solubility of 4.25 mg/ml in 20% PG/water.

Penetration Studies

Unless otherwise indicated, all penetration studies were conducted in duplicate and initiated using a known excess of the drug in the vehicle. The penetration data obtained from all studies were tabulated and analyzed by plotting the mean amount penetrated per unit area (Q) against time (T). The regression analyses of the plots were performed and 95% confidence limits of the steady-state regions of the penetration curve were established using a programmable calculator.[15] The regression lines were extrapolated to the time axis to determine the intercepts.

In order to overcome the variation due to skin samples from different donors and to effectively compare the results of various experiments, a standard control containing 20 mg/ml of benzocaine in 20/80 PG/water was included in all major experiments. The observed steady-state fluxes of the control ranged from a low of 25.17 $mcg/cm^2/hr$ to a

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[15] TI Programmable 59, Texas Instruments, Dallas TX

high of 64.57 mcg/cm^2/hr with an average of 42.16 mcg/cm^2/hr. This variation in flux values for the same control is mostly attributable to the variability in human skin samples from different donors and may be due to any number of factors such as skin age, skin condition, ethnic differences and skin metabolism (8). This variability has been considered in the design of all experiments.

DISCUSSION

To assure penetration of a drug from a vehicle, one approach utilizes the differences in thermodynamic activities of the penetrant between the vehicle and the membrane phases. Ideally, such vehicles "push" the drug into the skin without causing detectable injury to the skin membrane. Intuitively one would choose a vehicle that results in a relatively high thermodynamic activity of the active ingredient in the formulation. In the study by Lam (29) this was accomplished by using a near-saturated solution of benzocaine. However, benzocaine concentration in the applied vehicle suffered significant decline over the duration of the experiment (12-24 hours), making it difficult to interpret the data. This problem was overcome in the present study by maintaining constant thermodynamic flux in the donor vehicle with an excess of drug.

PG/Water System

The effect of benzocaine concentration on its penetration profiles from 20/80 PG/water is shown in Table II and Figure 2. Five different concentrations, covering a wide range above and below the saturation solubility of the drug in 20/80 PG/water system (4.25 mg/ml), were chosen to study the effect of benzocaine concentration. The steady state fluxes were found to increase significantly with increasing

TABLE II

The Penetration Pattern of Benzocaine from 20/80 PG/Water Gel as a Function of Its Concentration

Benzocaine Concentration (mg/ml)	Flux ($Mcg/Cm^2/Hr$)	Correlation Coefficient (r)	Intercept (Hrs)
1	12.13 (±1.26)	0.992	-2.70
3	20.55 (±2.40)	0.990	-2.50
5	29.51 (±2.64)	0.994	-2.02
10	36.61 (±2.46)	0.997	-1.42
20	37.23 (±1.29)	0.999	-0.29

Fluxes increased significantly with increasing drug concentration from 1 to 10 mg/ml ($P < 0.01$).

There was no significant difference in the fluxes of 10 and 20 ($P > 0.50$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

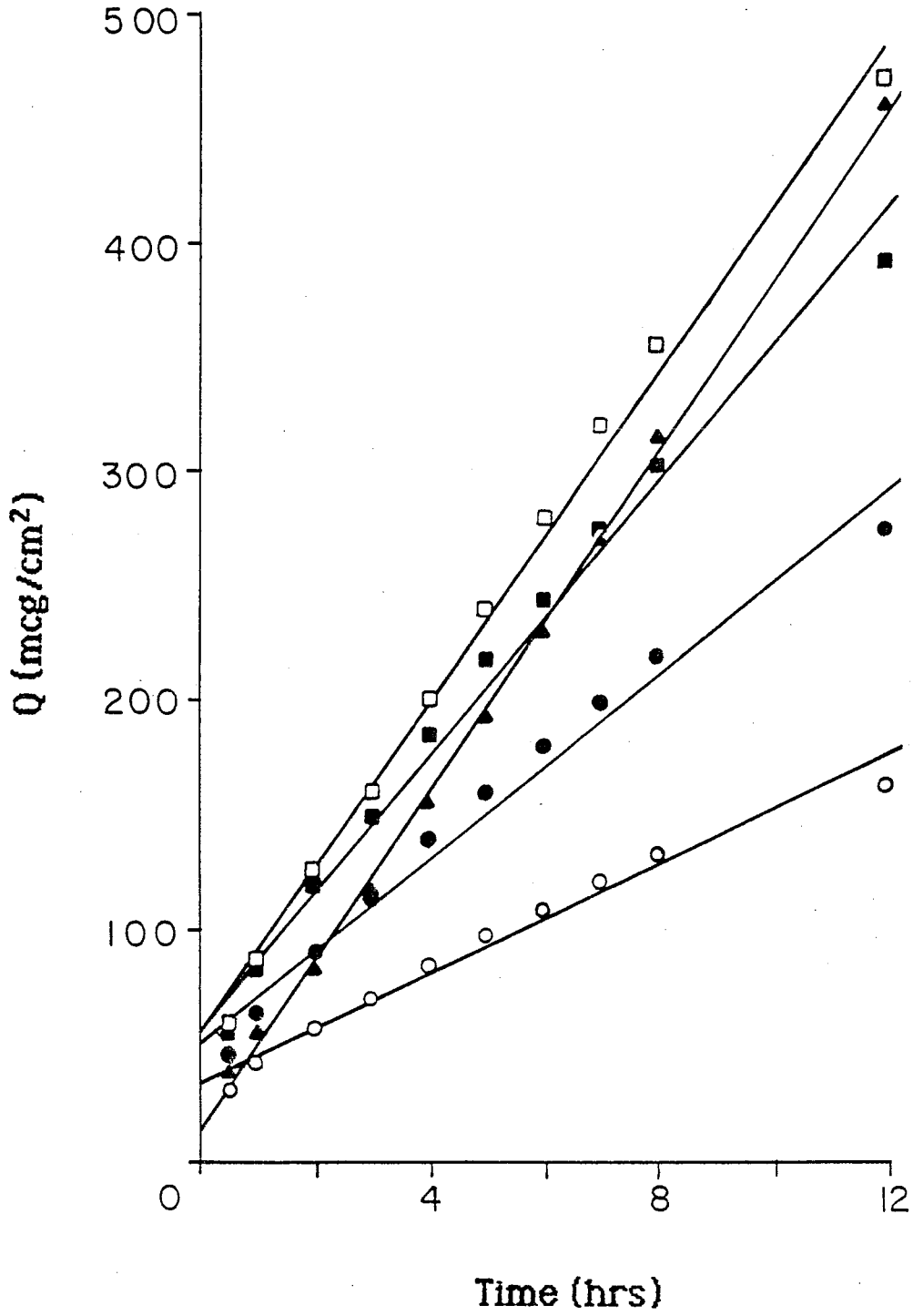

Figure 2. The Penetration Pattern of Benzocaine from 20/80 PG/Water Gel as a Function of its Concentration. Q versus t plot.

Key: Drug conc. (mg/ml) ○ 1 ● 3 ■ 5 □ 10 ▲ 20

drug concentration up to 10 mg/ml. At this point, doubling the drug concentration did not cause a significant change in its penetration rate.

Azone/PG/Water System

A preliminary study examined the penetration pattern of benzocaine at concentration levels of 3, 5 and 10 mg/ml in 20/80 PG/water gel containing 5% Azone (Table III, Fig. 3). Approximately 30% decline in the flux associated with a drop in drug concentration from 5 to 3% in the absence of Azone (Table II) is completely reversed in the presence of 5% Azone. The fluxes for both 3 and 5% benzocaine were almost equal. The presence of Azone in the formulation, at a benzocaine level below its saturation solubility in the vehicle, apparently helped to overcome the thermodynamic deficit resulting from very low drug concentration, therefore enhancing the penetration through the skin.

On the basis of Lam's work (29), three Azone concentrations (1, 5 and 10%) were chosen for the penetration study conducted at the benzocaine concentration of 20 mg/ml. As can be seen From Table IV and Fig. 4, a statistically significant increase in the flux ($P < 0.05$) relative to control resulted from applying the system containing 1% Azone. The delivery systems containing 5% and 10% Azone did not significantly enhance the cutaneous penetration of

TABLE III

The Penetration Pattern of Benzocaine from PG/Water 20/80 Gel Containing 5% V/V Azone

Benzocaine Concentration (mg/ml)	Flux ($Mcg/Cm^2/Hr$)	Correlation Coefficient (r)	Intercept (Hrs)
3	10.21 (±1.14)	0.991	-3.27
5	9.94 (±0.95)	0.993	-2.70
10	12.80 (±0.67)	0.998	-2.32

The flux from 3 mg/ml drug level showed no significant different as compared to that of 5 mg/ml level ($P > 0.20$).

There was a significant difference in the fluxes of 5 and 10 mg/ml level ($P < 0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

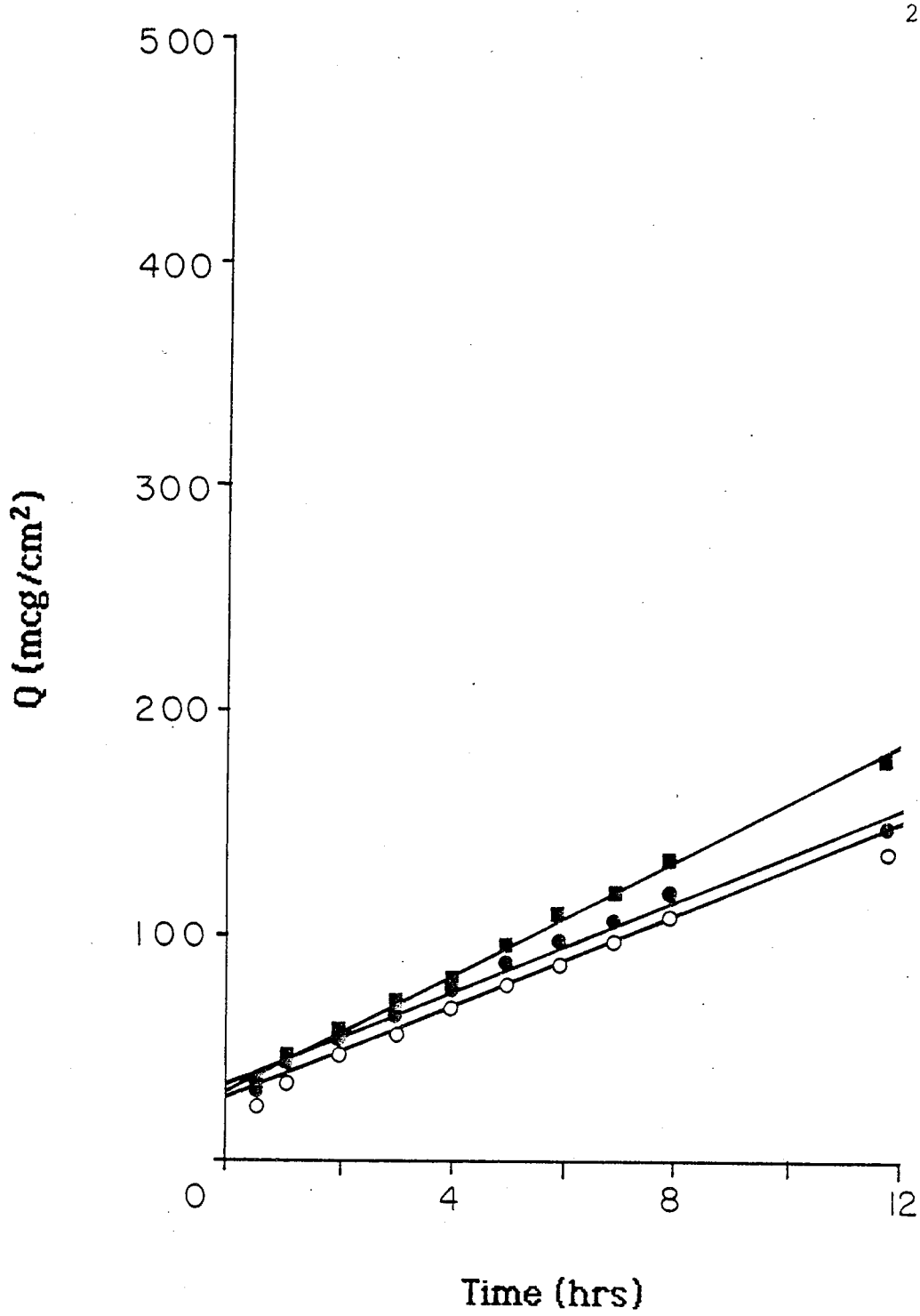

Figure 3. The Penetration Pattern of Benzocaine from PG/Water 20/80 Gel Containing 5% V/V Azone. Q versus t plot.

Key: Drug conc. (mg/ml) ● 3 ○ 5 ■ 10

TABLE IV

Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water at Drug Level of 20 mg/ml

Azone Concentration (% V/V)	Flux ($Mcg/Cm^2/Hr$)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
10	26.60 (±1.80)	1.06	0.995	-1.86
5	26.25 (±1.44)	1.04	0.997	-1.61
1	28.17 (±1.62)	1.12	0.996	-1.42
Control-1	25.17 (±1.21)	1	0.997	-1.82

Control-1 = 20 mg/ml benzocaine suspension in 20/80 PG/water gel.

The fluxes obtained from systems containing 5 and 10% Azone showed no significant difference as compared to control ($P > 0.10$) but flux from system containing 1% Azone was significantly different from that of control ($P < 0.05$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

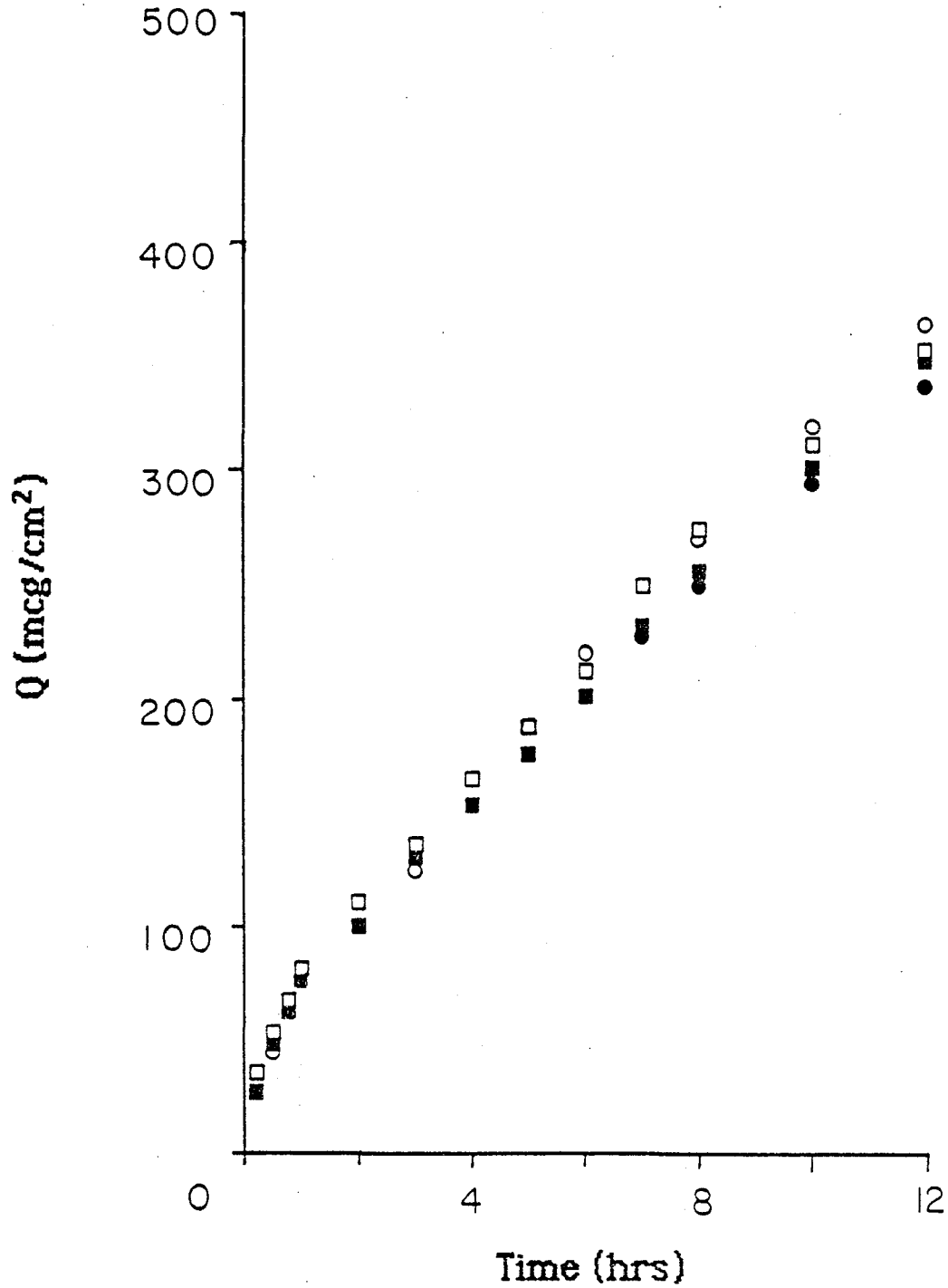

Figure 4. Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel at Drug Level of 20 mg/ml. Q versus t plot.

Key: Azone conc. (V/V) □ 10% ■ 5% ○ 1% ● Control

benzocaine. When the experiment was repeated under identical conditions at benzocaine concentration level of 10 mg/ml, the drug penetration rate from the vehicle with 1% Azone was approximately the same as the control; however, benzocaine flux was found to decrease significantly ($P < 0.01$) with increasing Azone concentration (Table V, Fig. 5). In yet another study under similar conditions (Table VI, Fig. 6), when initial benzocaine concentration of the four formulations (including control) was adjusted to twice the respective total solubility, the corresponding fluxes from the formulations containing 1 and 10% Azone were significantly greater ($P < 0.01$) than that of the control. The flux from the vehicle containing 5% Azone was very significantly different (higher) ($P < 0.001$) from the control value and was also higher than the flux value for vehicle with 10% Azone. These data reveal that skin penetration enhancement effects of Azone are a function of its concentration and there appears to be an optimum Azone level for obtaining maximum flux. The relative magnitude of the fluxes from the formulations containing 1, 5 and 10% Azone relative to control in each study can be explained by considering total drug concentration in each formulation in relation to its saturation solubility in the vehicle and taking into account that the solubility of benzocaine in Azone is approximately 60 times that in 20/80 PG/water, indicating a high affinity for benzocaine. Based upon the

TABLE V

Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel at Drug Level of 10 mg/ml

Azone Concentration (% V/V)	Flux ($Mcg/Cm^2/Hr$)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
10	10.13 (±1.05)	0.45	0.992	-3.06
5	12.80 (±0.67)	0.57	0.998	-2.32
1	22.96 (±1.12)	1.02	0.998	-0.84
Control-1	22.42 (±1.46)	1	0.997	-0.99

Control-1 = 10 mg/ml benzocaine suspension in 20/80 PG/water gel with no Azone.

Fluxes decreased significantly with increasing Azone concentration ($P < 0.01$).

There was no significant difference between the rates of control and 1% ($P > 0.20$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

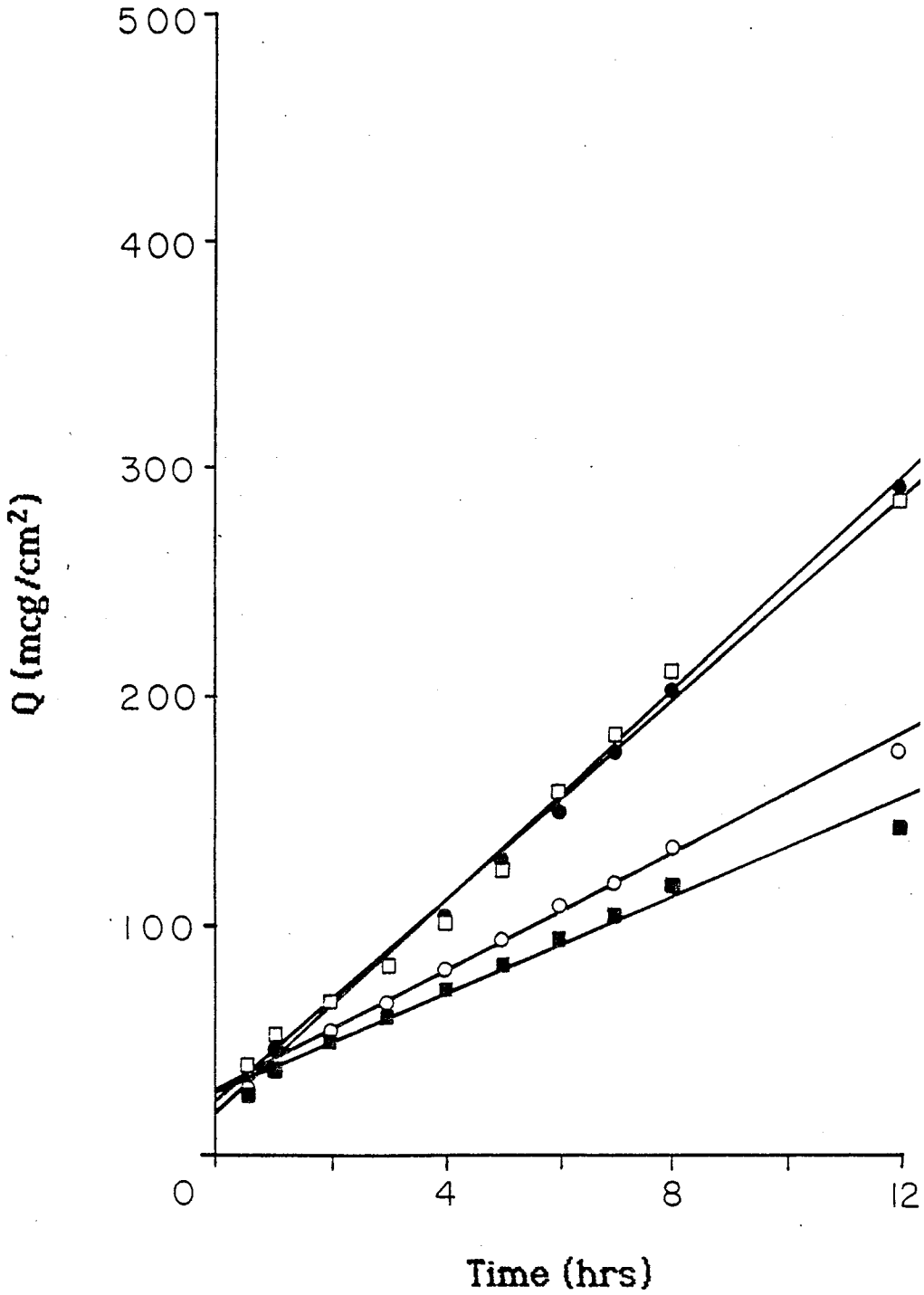

Figure 5. Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel at Drug Level of 10 mg/ml. Q versus t plot.

Key: Azone conc. (V/V) ■ 10% ○ 5% ● 1%
□ Control.

TABLE VI

Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gels

Azone Concentration (% V/V)	Benzocaine Concentration (mg/ml)	Flux (Mcg/Cm^2/Hr	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
10	60	21.15 (±1.97)	1.22	0.991	-6.93
5	35	24.91 (±2.01)	1.44	0.994	-7.24
1	15	22.24 (±2.07)	1.28	0.991	-6.08
Control-1	10	17.36 (±1.85)	1	0.989	-2.38

Control-1 = PG/water 20/80 gel containing 10 mg/ml drug.

Concentration of benzocaine in each system was double the saturation solubility.

The fluxes obtained from systems containing 1 and 10% Azone showed a highly significant difference from control ($P < 0.01$) and that from the system containing 5% Azone showed a very highly significant difference from that of control ($P < 0.001$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

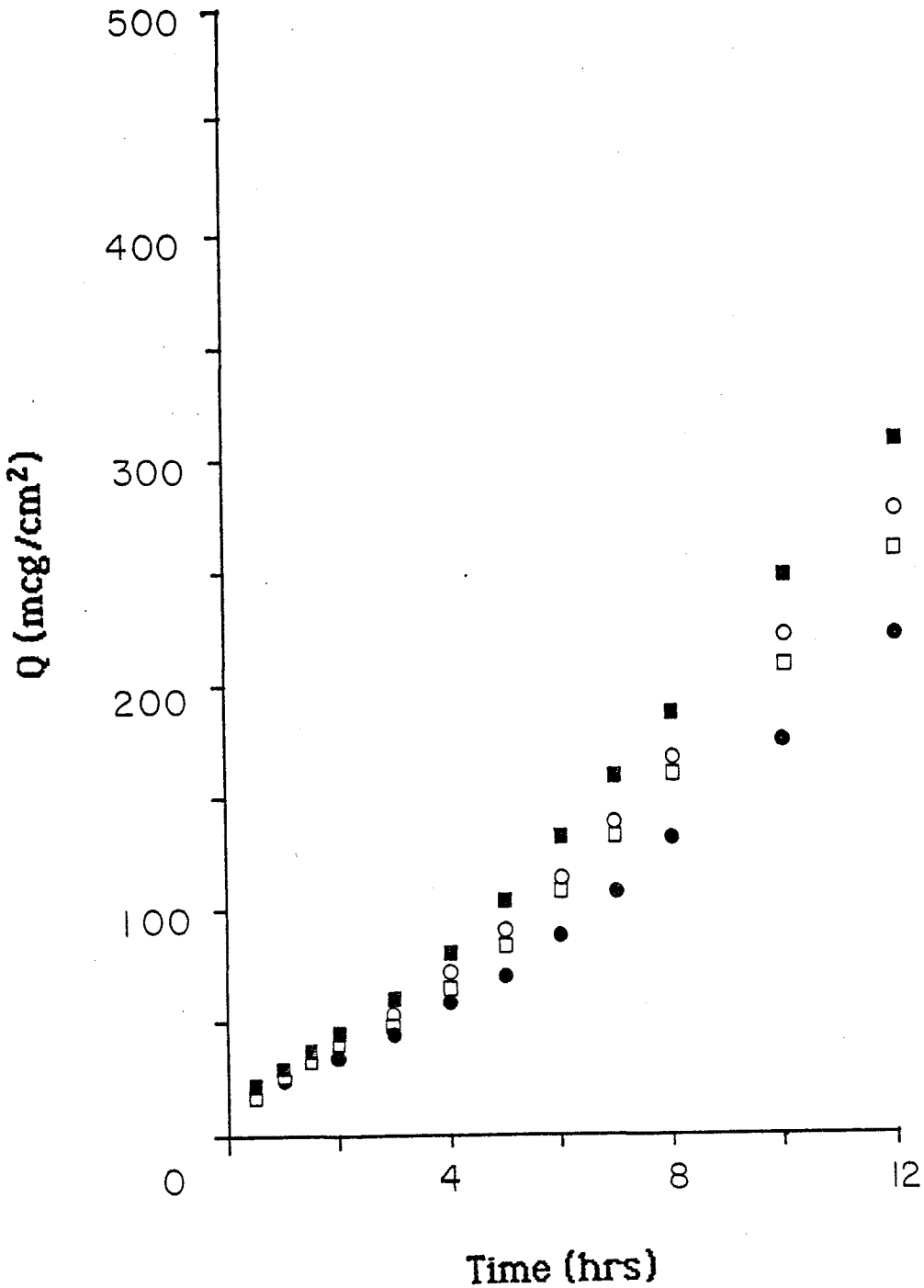

Figure 6. Effect of Azone Concentration on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gels. Q versus t plot.

Key: Azone conc. (V/V) □ 10% ■ 5% ○ 1%
● Control-1

solubility data, the saturation solubilities of benzocaine in the 20/80 PG/water formulations containing 1, 5 and 10% Azone were calculated to be 6.7, 16.5 and 29 mg/ml, respectively. The data for 10% Azone in Table IV suggest that drug depletion might have masked the penetration enhancement effect of Azone since the actual drug level of 20 mg/ml was well below the saturation level of 29 mg/ml. The plots of Figure 4 showing essentially a straight-line release over 12-hour duration negate this possibility. However, drug depletion effects do appear to contribute to the decline in flux when the drug concentration is cut in half to 10 mg/ml (Table V, Fig. 5). It is significant to note that flux declined when Azone concentration was increased from 5 to 10% even under conditions of constant thermodynamic potential (Table VI, Fig. 6). Collectively, the aforementioned observations lend strong support to the conclusion that benzocaine has a high degree of affinity for Azone and (at high levels of Azone) any penetration enhancement effects are strongly negated by a corresponding decrease in the skin/vehicle partition coefficient. Among the Azone concentrations investigated, 5% Azone generates maximum flux under conditions of constant thermodynamic activity of benzocaine in 20/80 PG/water gels. This work confirms the earlier findings of Lam (29) with respect to penetration effects of Azone on benzocaine incorporated in 60/40 PG/water gels at near-saturation level.

Effect of Azone Pretreatment of Human Cadaver Skin

This set of experiments was conducted to determine if the observed penetration enhancement effects of Azone were in part or whole due to any direct effects on the skin tissues. The basic strategy involved pretreating the excised human cadaver skin with neat Azone either by surface application or soaking prior to use in the drug penetration experiments. The surface treatment involved application of neat Azone to the epidermal surface of the skin mounted on the diffusion cell or soaking the skin sample in the neat Azone for an hour. In either case, after the removal of excess Azone, the skin was washed with ethanol followed by water and surface dried. The penetration profiles were then monitored. Two control preparations, with and without Azone, were used in these studies.

The penetration profiles of benzocaine following surface application of neat Azone for one, thirty and sixty minutes revealed that fluxes from the pretreated samples were significantly greater than either control (Table VII, Fig. 7) and were independent of the duration of exposure with the penetration enhancer. The results were similar when the presoaked and surface-treated samples were compared (Table VIII, Fig. 8). Together, these pretreatment studies reveal that Azone facilitates penetration of benzocaine molecules across human skin by directly reducing the barrier effect of stratum corneum. The water-rich dermal

TABLE VII

Effect of Azone Pretreatment (Surface) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel

Skin Condition	Flux (Mcg/Cm^2/Hr)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
1-min pretreatment	40.19 (±4.29)	1.32	0.989	-1.61
30-min pretreatment	43.12 (±3.51)	1.41	0.995	-1.51
1-hr pretreatment	41.67 (±3.56)	1.37	0.995	-1.82
Control[a]	30.51 (±1.19)	1	0.999	-0.99
Control[b]	34.40 (±4.01)	1.13	0.990	-2.44

Drug concentration in all preparations was 20 mg/ml.

[a]Control - No pretreatment.

[b]Control - No pretreatment. 5% Azone in the preparation.

The fluxes from pretreated skin were significantly different from controls ($P < 0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

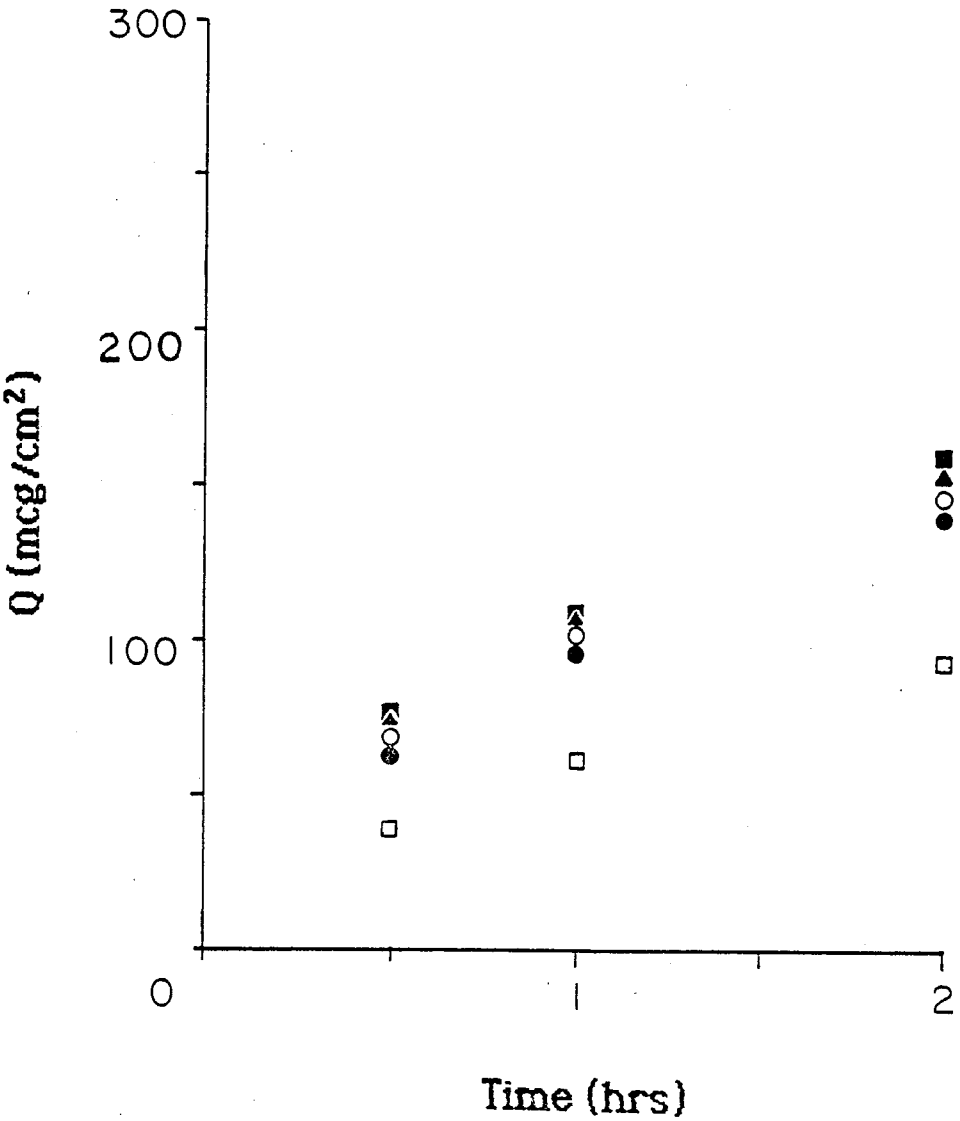

Figure 7a. Effect of Azone Pretreatment (Surface) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel. Q versus t plot.

Key: Treatment time (min.) ● 1 ○ 30 ■ 60
□ Control[a] ▲ Control[b]

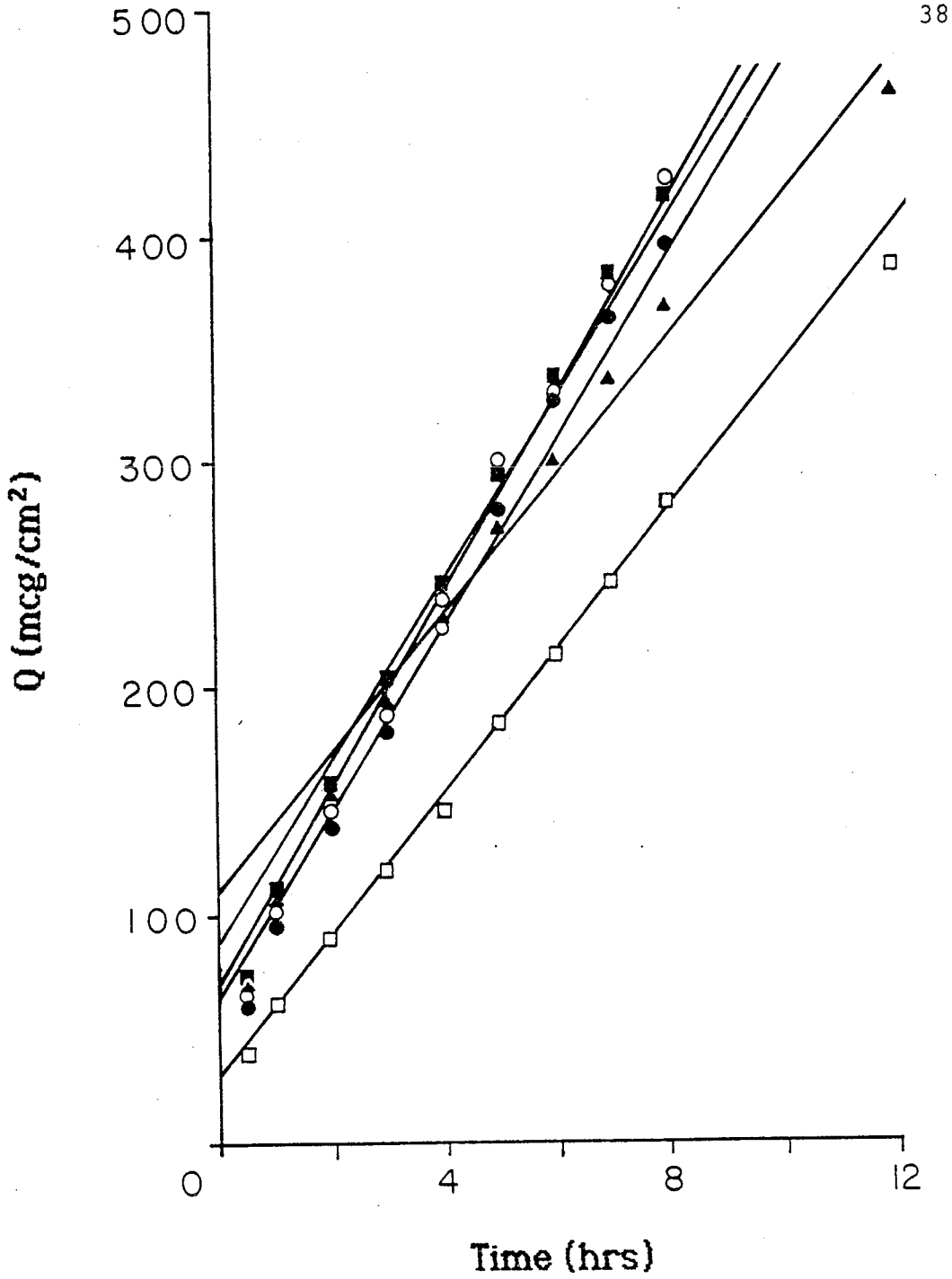

Figure 7b. Effect of Azone Pretreatment (Surface) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel. Q versus t plot.

Key: Treatment time (min.) ● 1 ○ 30 ■ 60
□ Control[a] ▲ Control[b]

TABLE VIII

Effect of Azone Pretreatment (Soaking Versus Surface Application) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel

Skin Condition	Flux (Mcg/Cm^2/Hr)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
Soaking	76.55 (±2.69)	1.19	0.999	-0.42
Surface Application	77.58 (±2.19)	1.25	0.999	-1.00
Control[1]	64.57 (±2.08)	1	0.999	-1.07
Control[2]	61.88 (±3.82)	0.96	0.999	-1.35

Benzocaine concentration in all preparations was 20 mg/ml.

Control[1] - No pretreatment. 20/80 PG/Water gel without Azone.

Control[2] - No pretreatment. 20/80 PG/Water gel with 5% Azone.

The fluxes from pretreated skin were significantly higher than controls ($P < 0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

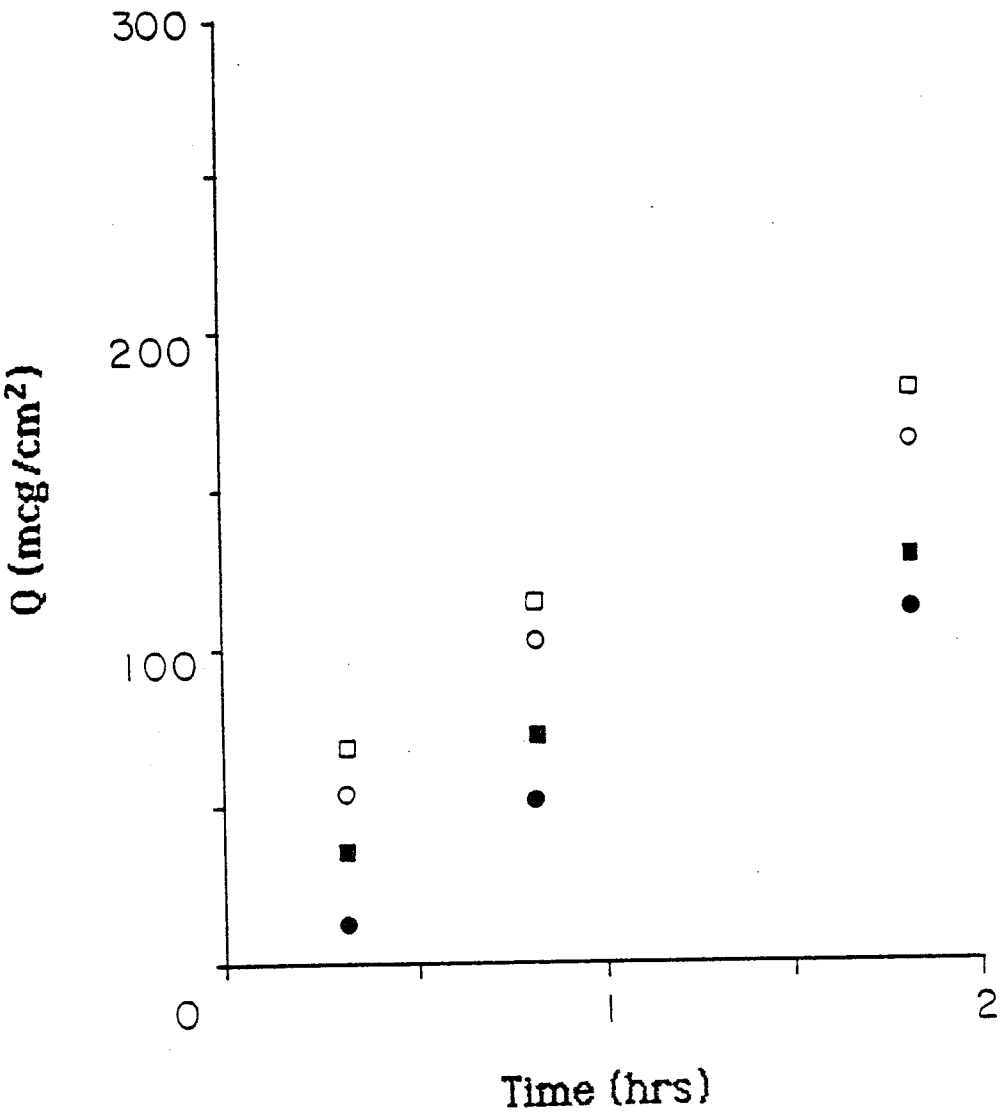

Figure 8a. Effect of Azone Pretreatment (Saoking Versus Surface Application) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel. Q versus t plot.

Key: ● Soaking ○ Surface treat. ■ Control[1] □ Control[2]

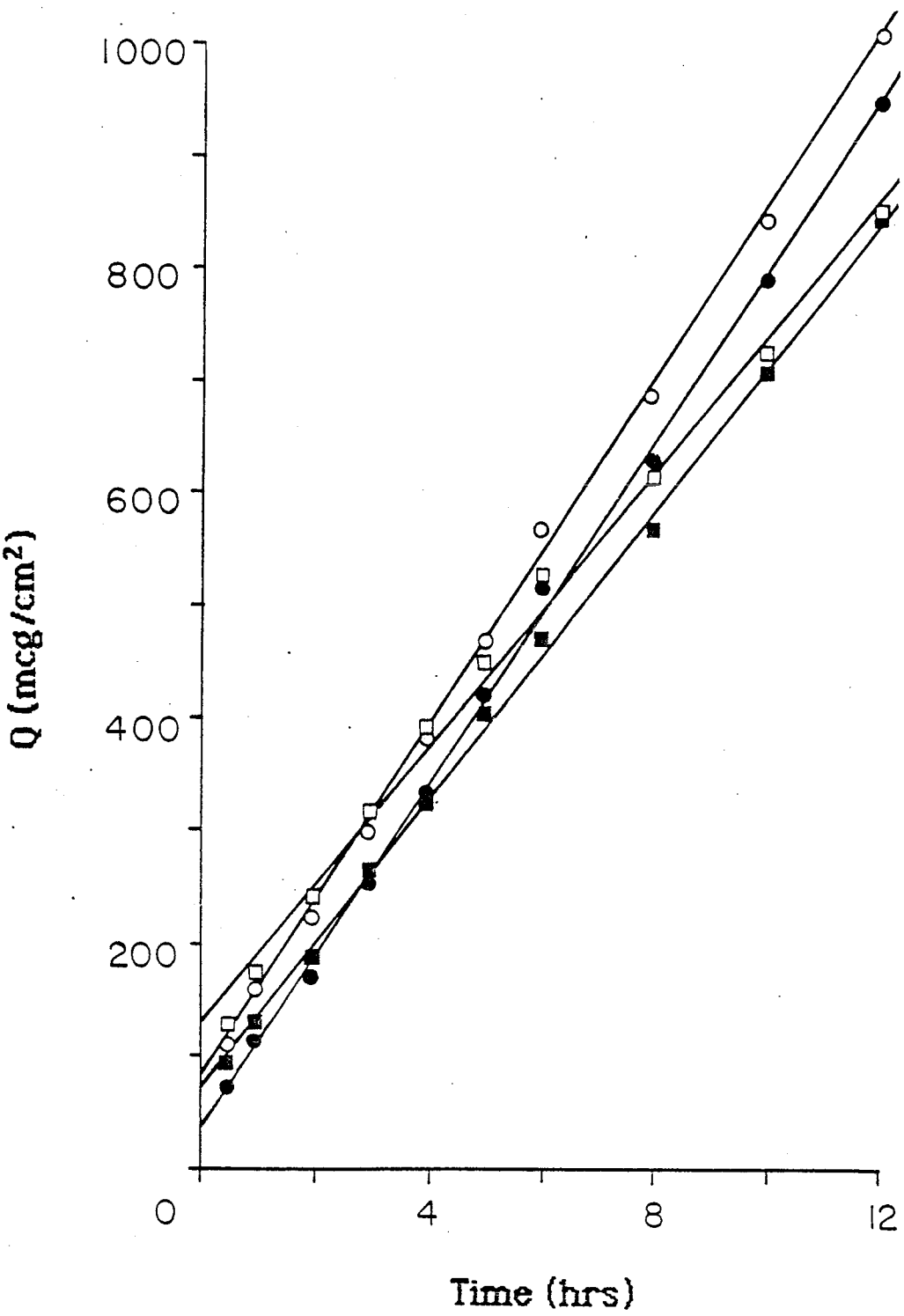

Figure 8b. Effect of Azone Pretreatment (Saoking Versus Surface Application) on Percutaneous Penetration of Benzocaine from 20/80 PG/Water Gel. Q versus t plot.

Key: ● Soaking ○ Surface treat. ■ Control[1] □ Control[2]

tissues do not appear to be involved. The barrier-lowering effect on the skin appears to be the primary component of the overall mechanism of penetration enhancement by Azone.

Effect of DMSO on the In-Vitro Percutaneous Penetration of Benzocaine

This study was designed to complement the work done earlier in our laboratory on the effects of DMSO on percutaneous absorption of benzocaine. The previous study (29) suggested that DMSO/water systems did not enhance the penetration of benzocaine into the stratum corneum when compared to the control of 60/40 propylene glycol/water. This was attributed in part to the drug depletion effects, and the increased solubility of benzocaine in DMSO/water system and consequent decrease in partitioning of benzocaine into the receptor skin.

The present study, conducted under the conditions of constant thermodynamic flux, suggested that DMSO significantly enhanced the penetration of benzocaine into the skin as compared to control (Table IX, Fig. 9). The steady-state fluxes were significantly increased with increasing DMSO concentration ($P < 0.01$). The DMSO-induced enhancement of penetration was observed over 40-80% range of concentration of DMSO in the vehicle relative to 60/40 PG/water control, also confirming that the lack of enhancement reported by Lam (29) was indeed due to drug depletion.

TABLE IX

Penetration of Benzocaine from DMSO/Water Systems Through Full Thickness Human Skin

DMSO/Water System (V/V)	Flux ($Mcg/Cm^2/Hr$)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
80/20	49.52 (±5.25)	1.62	0.990	0.95
60/40	42.87 (±3.22)	1.40	0.995	0.78
40/60	40.16 (±3.66)	1.31	0.993	0.35
Control	30.60 (±1.57)	1	0.998	0.44

Control = PG/water 60/40.

Concentration of benzocaine in all systems was above saturation throughout the duration of the experiment.

The fluxes of all DMSO/Water curves were significantly different from control ($P < 0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

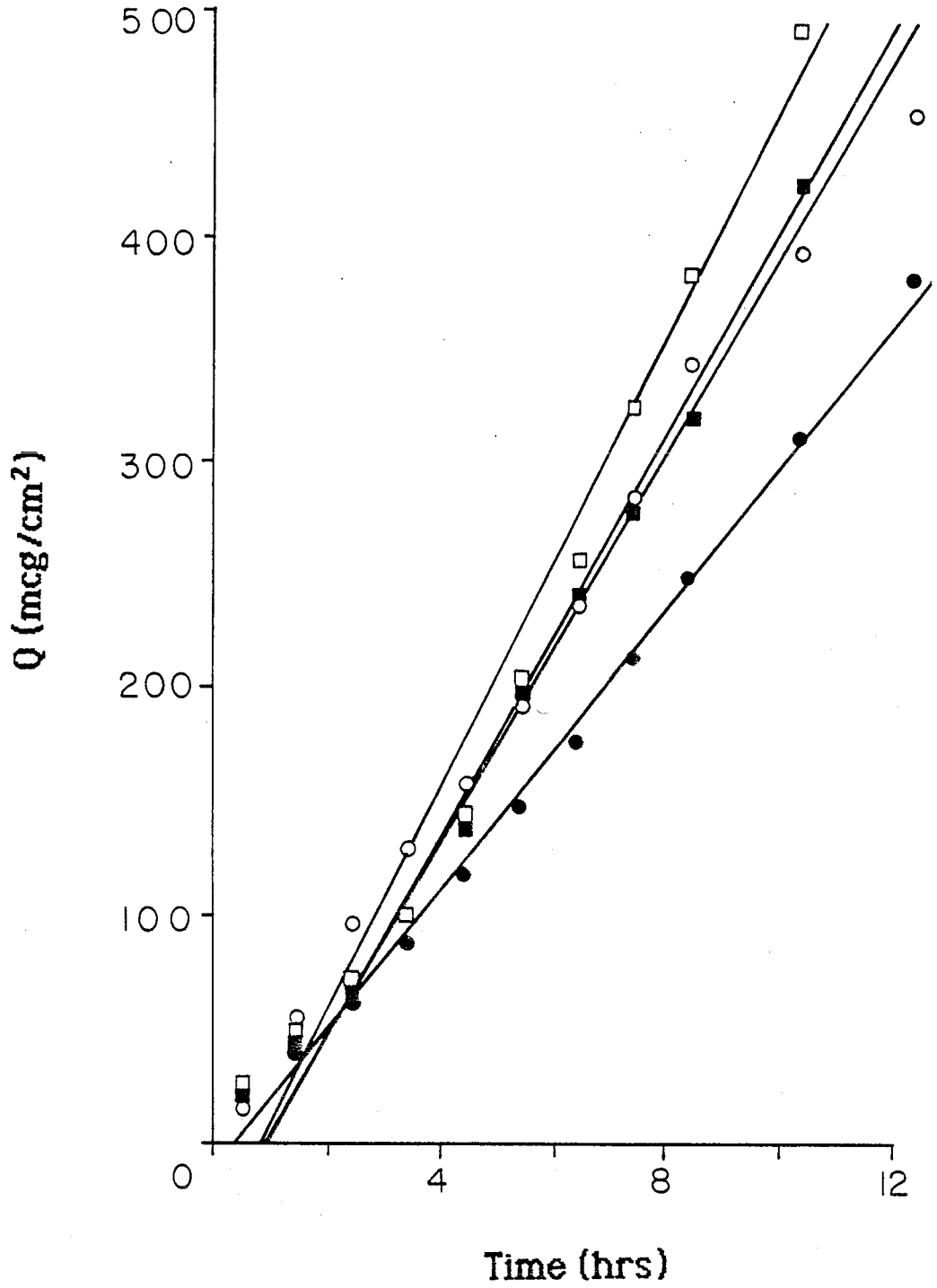

Figure 9. Penetration of Benzocaine from DMSO/Water Systems Through Full Thickness Human Skin. Q versus t plot.

Key: DMSO/water (V/V) □ 80/20 ■ 60/40 ○ 40/60 ● Control

Earlier reports (18,19,33) have suggested that DMSO concentration must be between 60 to 90% in order to see penetration enhancing effects.

Exposure of skin to pure DMSO (surface pretreatment) for one hour resulted in a significant increase in the penetration rate of benzocaine over controls a and c ($P < 0.01$) as shown in Table X, Figs. 10a and 10b. This suggested that observed penetration enhancement might be due to a direct effect of the solvent on the stratum corneum. The pretreatment of the skin with DMSO resulted in an increase of approximately 50% in the total amount penetrated per unit area in the initial hour relative to control-1, declining to approximately 42% after six hours, then to only 20% after twelve hours. Apparently DMSO exerts a significant but temporary effect on the epidermal barrier. The effect lasts while the DMSO is held in the stratum corneum and then as it is absorbed or washed out, barrier function returns.

The effect of duration of exposure to DMSO was not investigated during this study.

Effect of 2-Pyrrolidone on the Percutaneous Penetration of Benzocaine

The effect of 2-pyrrolidone on the percutaneous penetration of benzocaine is summarized in Table XI, Fig. 11. The study was done using different mixtures of 2-pyrrolidone

TABLE X

Percutaneous Penetration of Benzocaine Through DMSO-Pretreated Skin

System Identification	Flux (Mcg/Cm^2/Hr)	Correlation Coefficient (r)	Intercept (Hrs)
DMSO Pretreatment	34.94 (±1.00)	0.999	-1.33
Control[a]	29.48 (±2.30)	0.994	-0.58
Control[b]	34.48 (±1.94)	0.997	-0.22
Control[c]	27.70 (±1.56)	0.997	-1.03

[a]Benzocaine suspension in PG/Water 60/40.

[b]Benzocaine suspension in PG/Water 20/80.

[c]Benzocaine suspension in water. It was applied after pretreatment.

The fluxes through pretreated skin were significantly different from those of controls a and c ($P < 0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

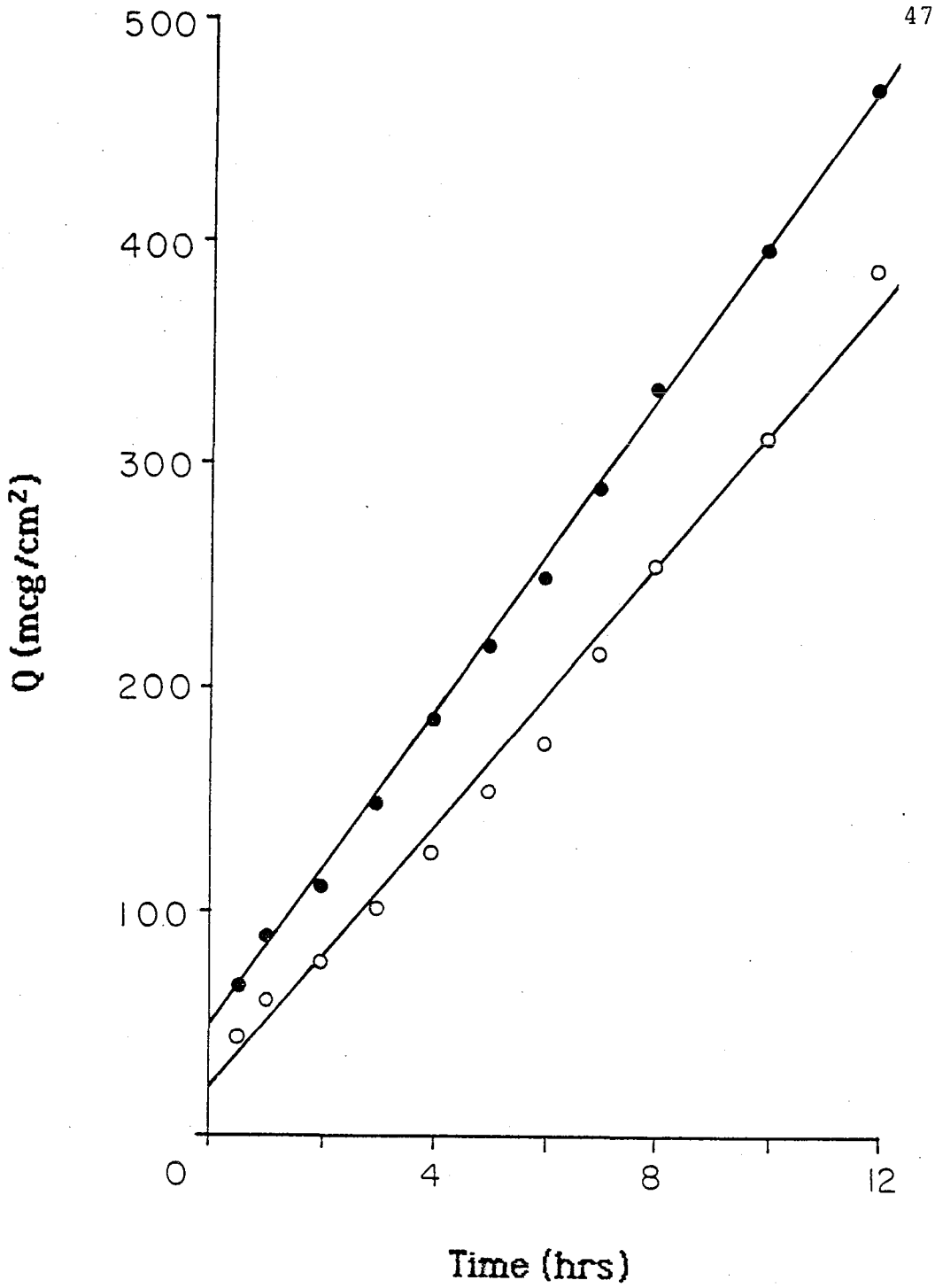

Figure 10a. Percutaneous Penetration of Benzocaine Through DMSO-Pretreated Skin. Q versus t plot.

Key: ● DMSO pretreatment
○ Control (60/40 PG/Water)

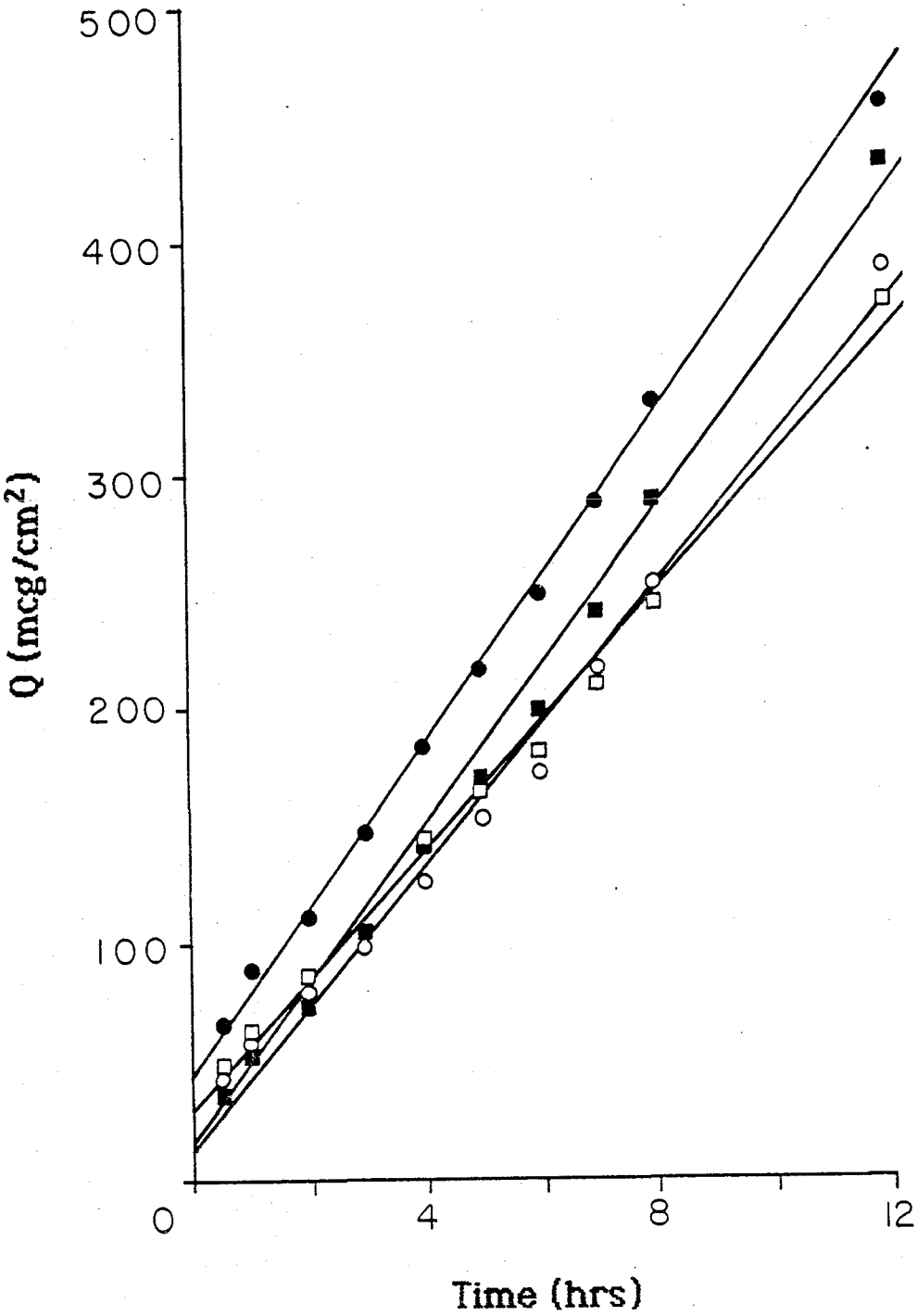

Figure 10b. Percutaneous Penetration of Benzocaine Through DMSO-Pretreated Skin. Q versus t plot.

Key: ● DMSO treatment ○ Control[a] ■ Control[b] □ Control[c]

TABLE XI

Effect of 2-Pyrrolidone Concentration on Percutaneous Penetration of Benzocaine Through Full Thickness Human Skin

Pyrrolidone/ Water System (% V/V)	Flux ($Mcg/Cm^2/Hr$)	Relative Rate	Correlation Coefficient (r)	Intercept (Hrs)
10	38.75 (±1.63)	0.69	0.999	-0.84
20	41.14 (±2.22)	0.73	0.998	-0.62
40	52.35 (±2.81)	0.93	0.998	-0.57
80	66.12 (±6.01)	1.18	0.994	-1.47
Control-1	56.05 (±0.68)	1	0.999	-0.62
Pre-treatment[a]	51.67 (±3.04)	0.92	0.997	0.59

Control-1 = 20 mg/ml drug in PG/Water 20/80 suspension.

Fluxes increased significantly as pyrrolidone concentrations increased ($P < 0.01$).

Pretreatment showed no significant enhancement of penetration over control.

The numbers in parentheses indicate 95% confidence limits of the respective slope.

[a]The skin was surface-pretreated with neat 2-P for one hour.

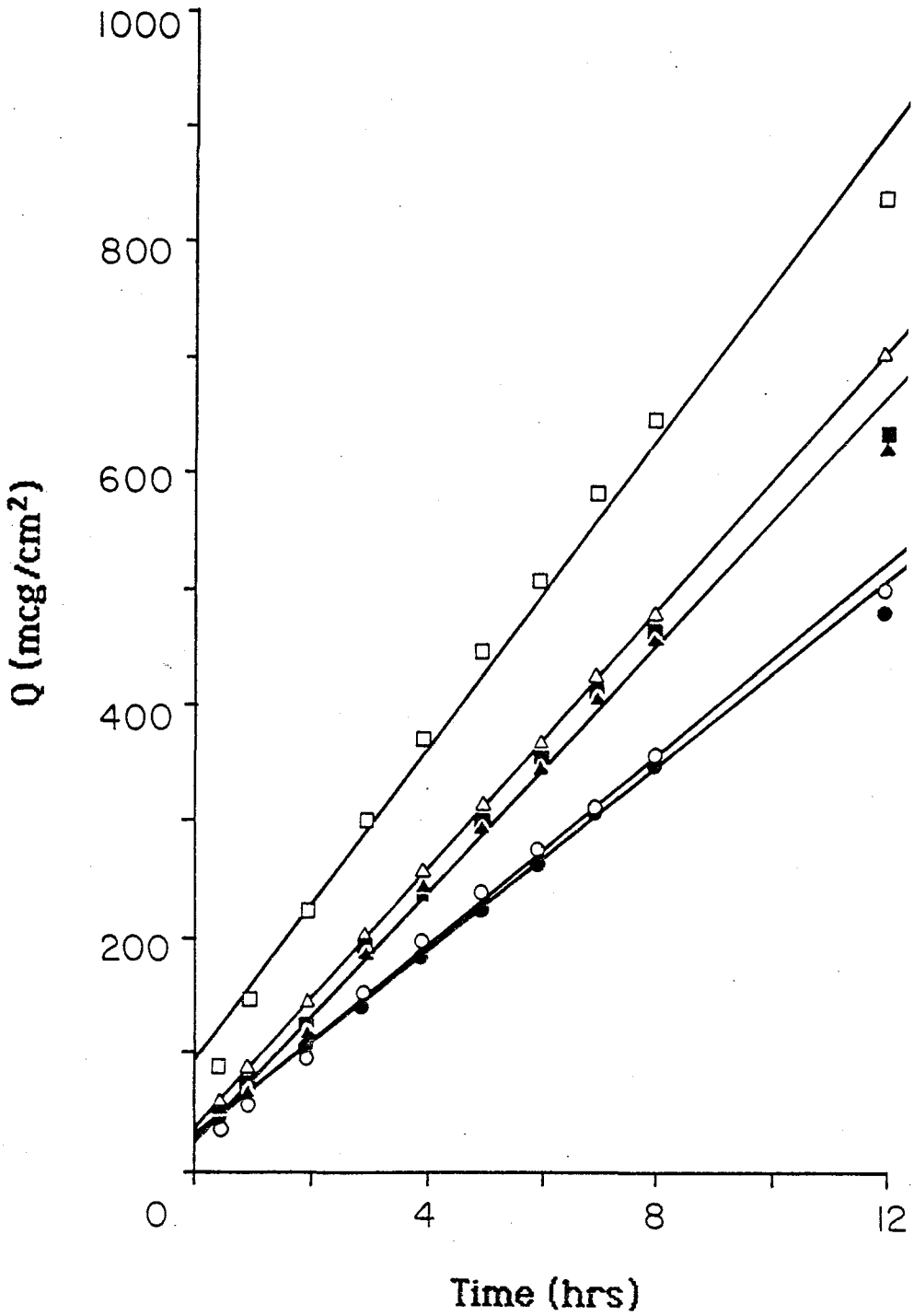

Figure 11. Effect of 2-Pyrrolidone Concentration on Percutaneous Penetration of Benzocaine Through Full Thickness Human Skin. Q versus t plot.

Key: Pyrrolidone conc. (V/V) ● 10% ○ 20% ■ 40% □ 80% ▲ Pretreatment △ Control

in water (10, 20, 40 and 80% V/V) to study its concentration effect on benzocaine penetration and to also see if the observed enhancement, if any, was due to direct effect on the stratum corneum. The constant thermodynamic activity in the donor vehicle was ensured by adding drug in excess of the solubility in each vehicle.

As can be seen from the results, the steady-state fluxes increased significantly ($P < 0.01$) with increasing pyrrolidone concentration. However, 2-pyrrolidone showed measurable enhancement over the control only at 80% V/V concentration. Furthermore, pretreatment of the skin with 2-pyrrolidone by surface application for an hour produced no significant enhancement of benzocaine penetration over the control. Berry (24) has shown that 2-pyrrolidone behaves similarly to the aprotic solvent DMSO in the presence of small polar penetrants such as methanol; however, its effect on larger, more complex semi-polar or nonpolar molecules may not be easy to delineate. In the same study, the permeability coefficient, stratum corneum/vehicle partition coefficient and diffusion coefficient of both caffeine and octanol were shown to decrease in the presence of 2-pyrrolidone. The moderate enhancement of benzocaine penetration observed in this study in the presence of 80% 2-pyrrolidone in water could be due to decrease in diffusional resistance of the stratum corneum brought on by a slow interaction with stratum corneum.

Additional studies including 2-pyrrolidone pretreatment, covering longer duration of exposure to the solvent, are needed in order to fully understand 2-pyrrolidone effect on percutaneous penetration of benzocaine and to draw meaningful conclusions.

SUMMARY AND CONCLUSION

The reported lack of efficacy of many OTC products containing benzocaine during clinical use has encouraged continuing efforts towards improving the percutaneous penetration of benzocaine. The present study combined the thermodynamic approach with the incorporation of various adjuvants in an attempt to maximize the in vitro percutaneous penetration of benzocaine. The strategy involved formulating each vehicle so as to sustain the optimum thermodynamic potential of the drug across the donor vehicle and receptor skin over a long period while maintaining a relatively high rate of drug transfer. The role of the so-called "penetration enhancers" Azone and dimethylsulfoxide was investigated in this context. 2-Pyrrolidone was also briefly examined in view of the recent encouraging reports (24,25).

Azone demonstrated a concentration-dependent enhancement of benzocaine penetration through skin from Azone/propylene glycol/water systems containing 1, 5 and 10% Azone respectively. At low drug levels (below saturation solubility), the incorporation of Azone in the formulation effectively compensated for the thermodynamic deficit. At high drug levels (drug in excess of solubility) 5% Azone appeared to have the maximum effect on the steady-state

fluxes from 20/80 PG/water system. Pretreatment studies demonstrated that penetration enhancement by Azone was significant, long lasting and largely due to a direct barrier-lowering effect on the stratum corneum. Benzocaine is extremely soluble in Azone. It would appear that the major penetration-enhancing effect of Azone is strongly negated by a reduction in vehicle to skin partitioning due to its high affinity for benzocaine.

Under the conditions of the experiment, dimethylsulfoxide enhanced the percutaneous penetration of benzocaine through full-thickness human skin at a concentration level of 40-80% in water. Pretreatment studies suggested that DMSO exerted a significant but temporary effect on the skin barrier.

With 2-pyrrolidone, a significant enhancement over control was seen only at a very high 2-P level (80%). The results of the pretreatment studies were not conclusive. A direct barrier-lowering effect on the skin, if present, is relatively slow to take effect. Further investigation is warranted.

In general, the three penetration enhancers examined have demonstrated significant penetration enhancement of benzocaine across human cadaver skin; however, the effects were not dramatic. Benzocaine readily penetrated the well-hydrated skin from the PG/water vehicle. The incorporation of Azone into this system might not be the most

effective way to take advantage of its considerable barrier-lowering effect on stratum corneum, especially for hydrophobic drugs like benzocaine. With molecules of much lower intrinsic penetrability and high hydrophilicity, Azone might produce dramatic effects. The clinical potential for benzocaine formulations containing DMSO would appear to be limited due to its high concentration requirement and consequent possibilities for side effects. Although 2-P did not show promise with benzocaine it deserves further screening with a spectrum of drugs of different physical/chemical characteristics in order to assess its full potential as adjuvant in dermatologic formulations.

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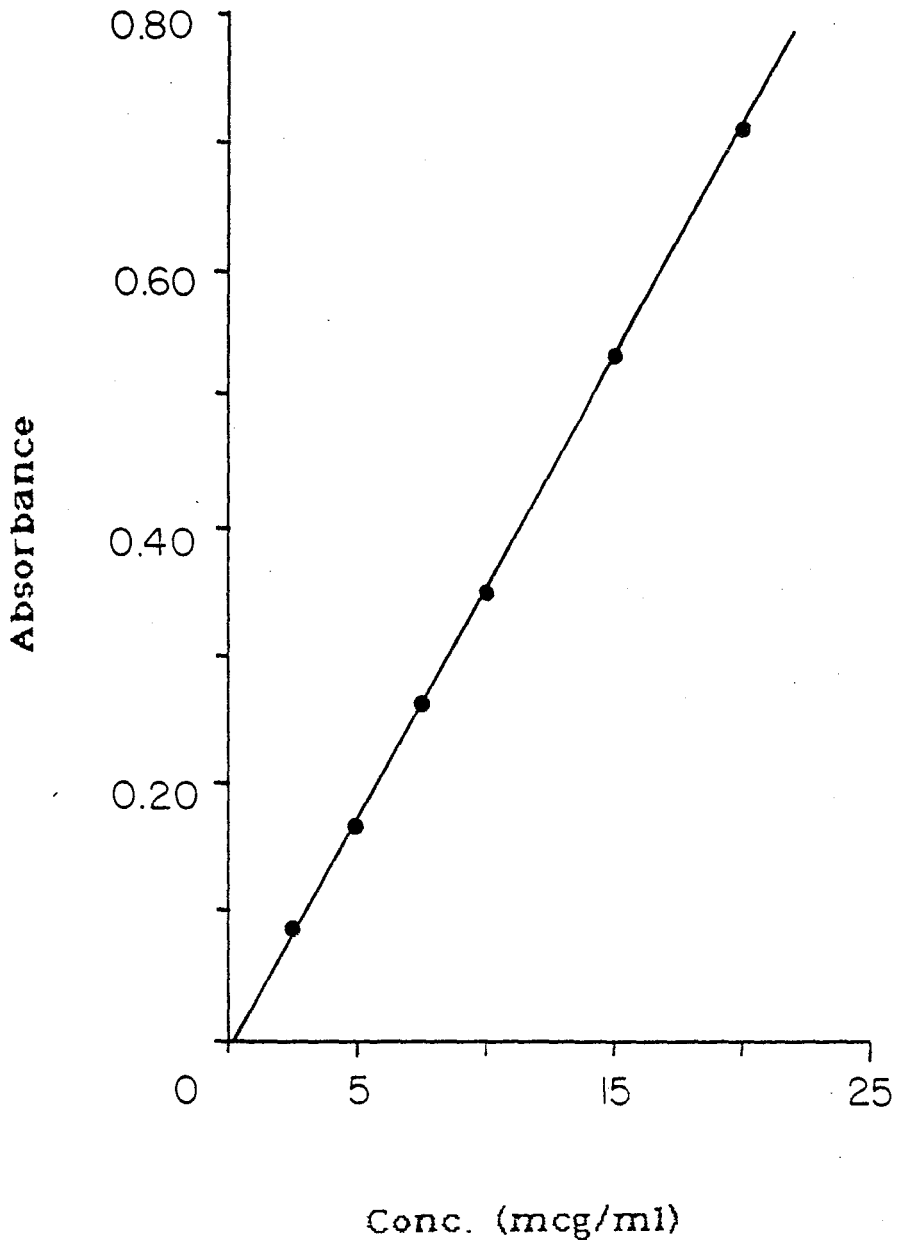
APPENDIX A

Beer's Law Plot of Benzocaine in Normal Saline



APPENDIX B

Beer's Law Plot of Benzocaine in Ethanol