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Investigation Of Physicochemical And Formulation Parameters For Transdermal Delivery Of Isoproterenol HC1

Rajesh Arvindkumar Patel
University of the Pacific

INVESTIGATION OF PHYSICOCHEMICAL AND FORMULATION PARAMETERS FOR TRANSDERMAL DELIVERY OF ISOPROTERENOL HCL

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Rajesh Arvindkumar Patel

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ABSTRACT OF DISSERTATION

Effects of solubility, partition coefficient, pH and selected adjuvants (propylene glycol and Azone) on the percutaneous penetration of isoproterenol HCl *in vitro* have been investigated using human cadaver skin. Preliminary stability studies demonstrated that isoproterenol HCl was very stable (< 1% decomposition) for 24 hours at 22° ± 0.5° in the pH range 1 to 7 in the following solvents: water, normal saline, propylene glycol and a series of propylene glycol-water mixtures (10,20,40 and 60 % v/v). The rate of decomposition of the drug in aqueous solutions increased with pH beyond pH 8. In normal saline, the decomposition was significant when the temperature was raised to 37° ± 0.5°. The solubility of isoproterenol HCl decreased and its skin/vehicle partition coefficient increased with increasing proportions of propylene glycol in the vehicle.

Results of the physicochemical and percutaneous penetration studies revealed that 20% v/v propylene glycol in water should be the optimal vehicle for transdermal delivery of isoproterenol HCl. Optimal penetration enhancing effects of Azone^{R} were seen when incorporated at a concentration of 1% v/v in the 20% v/v propylene glycol-water vehicle and more dramatically when the skin was pretreated with pure Azone for 60 minutes prior to application of the drug formulation. The flux reached a maximum around pH 9 in agreement with the predicted

favorable pH environment for neutral forms of isoproterenol HCl. Both neutral and charged forms of isoproterenol HCl were found to contribute to the total flux in agreement with the proposed model: $J = (Kp . C)^{ca} + (Kp . C)^{an} + (Kp . C)^{n}$. The calculated permeability coefficients for isoproterenol HCl were 0.2098×10^{-3}, 0.1570×10^{-4} and -0.8665×10^{-3} cm/h for neutral, cationic and anionic species, respectively. Azone enhanced the penetration of all forms of isoproterenol HCl, although the effect was more pronounced on the anionic species. This may be due to facilitation of penetration by formation of an ion-pair between isoproterenol HCl and Azone. The permeability coefficients for the neutral, cationic and anionic forms of isoproterenol HCl for penetration through Azone-pretreated skin were 0.8395×10^{-3}, 0.1701×10^{-3} and 0.8091×10^{-2} cm/h, respectively.

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This thesis is dedicated to my mother and to the memories of my father whose encouragement and support have contributed immeasurably to my professional growth and development.

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INTRODUCTION

Transdermal drug delivery systems (1) for systemic medication are one of the most promising recent innovations developed by the pharmaceutical industry. Research on transdermal drug administration for the systemic delivery of drugs has become very popular because it offers the potential for:

1. reducing frequency of dosing for drugs with short biological half lives,
2. increasing patient compliance,
3. reducing first-pass metabolism,
4. avoiding the influence of passage through gastrointestinal tract, and
5. controlling onset and termination of therapy.

The feasibility of this route of drug administration has been successfully demonstrated by introduction of products such as Transderm-Scop^{R}, a transdermal scopolamine product (2,3), Catapress TTS^{R}, a transdermal clonidine product (4-6) and various transdermal nitroglycerin products (7), Nitro-Dur^{R}, Transderm Nitro^{R}, Nitrodisc^{R} and Deponit^{R} (8-11).

The designing of an effective transdermal delivery

system is a real challenge for a pharmaceutical scientist because of the excellent barrier properties of the skin (12,13). Although the skin is one of the largest organs in the body and the most readily accessible for application of drugs, its surface is one of the least permeable as it functions to protect the body from the external environment. The skin is essentially composed of three main parts, epidermis, dermis and subcutaneous tissue. The principle barrier to drug penetration has been shown to be the epidermis. Of the various layers of epidermis, the stratum corneum, which is the outermost layer, offers the greatest resistance to drug penetration (14). It is a thin, ultradense polyphasic epidermal layer made from dehydrated highly filamented dead cells which are fused into a tight columnar arrangement. This layer has regions rich in protein separated by lipoidal framework. In its normal state, the stratum corneum contains 15% water. Beneath the stratum corneum, the living epidermis is much more hydrophilic in nature. The diffusional properties of this region can be considered to be similar to those of an aqueous protein gel (12). Beneath this lies the dermis, a region highly perfused by blood and lymph vessels. This microcirculation network provides an efficient means of drug removal into the systemic circulation. The composition of the various

layers of skin largely explains its varying degrees of penetrability. Each penetrant may encounter a different type of barrier depending upon its chemical and physical properties and potential interaction with the tissues.

Early investigations have shown that drugs to be formulated into transdermal products must be stable, preferably have a molecular weight of less than 1000 and melting point of less than 200° F, possess solubility in both hydrophilic and lipophilic solvents and have pH of 5-9 in saturated aqueous systems (13). These properties affect the most essential characteristic of the drug, namely, its permeation across the skin in therapeutically potent amounts over an acceptable area. Another requirement for acceptability is the drug's freedom from localized irritating or allergenic effects. In practice, very few drugs could meet all of these criteria and a variety of approaches must be tried in order to successfully formulate poorly penetrating drugs into transdermal delivery systems.

Early attempts to improve percutaneous penetration of weakly penetrating drugs focused primarily upon suitably altering penetrant solubility (15) and skin-vehicle partitioning (16) by judiciously blending two or more solvents (17,18). Poulsen et al. (19) and Ostrenga et al. (20) successfully demonstrated the

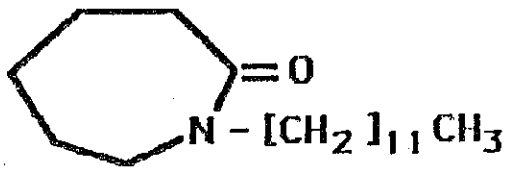
application of this approach to the formulation of a dermatologic preparation for fluocinolone acetonide and its 21-acetate ester. Studies conducted by Coldman et al. (21) have shown that percutaneous penetration of corticosteroids could be improved by incorporating a volatile component in the vehicle. Recently Nagai et al. (22) reported that a gel ointment of 55% ethyl alcohol/ propylene glycol containing propranolol gave distinctly higher plasma levels as compared to conventional propranolol ointments when administered topically. Sarpotdar et al. (23) showed that polyethylene glycol 400 has a significant barrier specific effect on the penetration rates of compounds and that these effects were related to the alteration of skin structure and the mass flow of water. The effect of particle size and vehicle on the percutaneous penetration of fluocinolone acetonide was earlier investigated by Barrett et al. (24). They found that particle size plays an important role in percutaneous penetration of fluocinolone acetonide and that the incorporation of propylene glycol into the vehicle enhanced the percutaneous penetration of this steroid. Various other studies (25-27) have shown that the incorporation of propylene glycol into the vehicle increased percutaneous penetration of various compounds. These studies also revealed that propylene

glycol penetrates the skin rather easily and that the skin resistance to propylene glycol decreased as the weight fraction of propylene glycol was increased. Together the aforementioned studies strongly suggest that propylene glycol increases percutaneous penetration of drugs by reducing the diffusional resistance of the skin barrier and increasing the thermodynamic flux of the drug by increasing its solubility in the vehicle.

Various investigations (28-31) have documented the effect of pH in the absorption of ionizable drugs through the gastrointestinal tract. The unionized drug molecules have a better penetration rate compared to ionized molecules due to the lipophilic nature of the gastrointestinal membrane. Similar observations have been made in early percutaneous penetration studies (32,33) conducted with ionizable drugs. Adriani and Dalili (34) found that salts of local anesthetic agents have very poor penetrability through the skin. Arita et al. (33), in their investigation with salicylic acid as an acidic drug and carbinoxamine as basic drug, showed preferential penetration of the unionized form over ionized form. Swarbrick et al. (35) in their studies with ionizable compounds showed the influence of pH on percutaneous penetration of four related 4-oxo-4-H-1-benzopyran-2-carboxylic acids. It was reported that

chromone-2-carboxylic acids permeate the human skin both as unionized and ionized species, although the former are approximately 10^4 times more permeable. Recently, Vaidyanathan et al. (36) have studied the effect of pH and solubility on the skin penetration of methotrexate from a 50% (v/v) propylene glycol-water vehicle. A pH of 4 to 5 was shown to be the most favorable environment for percutaneous penetration of methotrexate since the concentration of the unionized species at that pH is optimal.

Another popular approach to improve percutaneous penetration of drugs focuses primarily upon the use of adjuvants to diminish the skin's resistance to penetration of drug molecules. The effect on the skin should be mild and/or reversible. A variety of compounds (37) including many surfactants (38-42), dimethyl sulfoxide (43), 2-pyrrolidone (44-50), N-methyl-2-pyrrolidone (46-49) and Azone^R (1-dodecylazacycloheptan-2-one) (Figure 1) have been extensively studied. Among these, 2-pyrrolidone has been the subject of the most recent reports. Although Chow (51) recently reported that Azone affects the cell proliferation of cultured human and mouse L-929 skin fibroblasts, Azone appears to have considerable potential because of its documented



1 - Dodecylazacycloheptan - 2- one

$C_{18}H_{35}NO$ F.W. = 281.49

Figure 1. Chemical structure of Azone®.

lack of irritation and side effects. Azone at concentrations of 1-10%, has been shown to enhance penetration of most of the drugs studied by a factor which may be as much as hundreds of times the control values (52,53). Several studies have attempted to evaluate the effect of Azone on the percutaneous penetration of triamcinolone acetonide (54,55), methanol (56), 8-bromoadenosine 3′, 5′- hydrogenphosphate, 9-B-D-arabinofuranosyladenine (57), arabinofuranosyl-adenine 5′-valerate (58), 5-fluorouracil (59,60), indomethacin and hydroquinone (59), hydrocortisone (50), mannitol (50), hexyl nicotinate (61), 6-carboxy-fluorescein (62), trifluorothymidine (63) and metronidazole (64). These studies have shown that Azone indeed enhances the percutaneous penetration of these drugs. Stoughton (54) reported that percutaneous penetration of triamcinolone acetonide through human skin in the presence of 10% Azone was 5-10 fold higher. Similar results were obtained by Chow et al. (55), when they investigated the percutaneous penetration of triamcinolone acetonide through hairless mouse skin. Greater enhancement was observed with 5-fluorouracil and indomethacin at Azone concentrations of 1.8-45% (59). In another study by Sugibayashi et al. (60), similar results were observed with 5-fluorouracil. Bennett and Berry

(50) showed that Azone in presence of propylene glycol enhanced the percutaneous penetration of polar and non-polar compounds. Behl et al. (56) demonstrated that the permeability of methanol was promoted 200-400 fold by Azone with maximal enhancement at 10-25%. Vaidyanathan et al. (57) showed an 170-fold increase in the penetration of 9-B-D-arabinofuranosyladenine over the control values. Enhanced skin penetration of hexyl nicotinate in presence of Azone in human subjects using laser Doppler velocimetry was demonstrated in a recent study by Ryatt et al. (61). Wotton et al. (64) observed prolonged enhancement of percutaneous penetration of metronidazole lasting over several days following single application of Azone. Ohshima et al. (62) have shown that Azone promotes the penetration of a poorly penetrating dye, 6-carboxyfluorescein. Sheth et al. (63) recently reported that Azone and propylene glycol enhance membrane permeability of trifluorothymidine and act synergistically when formulated together. Recently, BenKorah (65) has confirmed earlier findings of Lam (66) that Azone increases the skin penetration flux of benzocaine from selected propylene glycol/water vehicles by directly affecting the skin's barrier characteristics. It appears that for lipophilic drugs, such as benzocaine, drug solubility in Azone might adversely effect the

skin/vehicle partitioning of the drug. This would make selection of the proper Azone concentration level in the vehicle is of critical importance. Literature has very few citations regarding effect of Azone on percutaneous penetration of ionizable compounds. Recently, Hadgraft et al. (67) reported that Azone facilitates the transport of salicylate ion across an isopropyl myristate membrane by forming ion pairs. This would suggest that the selection of proper Azone concentration in the vehicle is also of critical importance for hydrophilic drugs as Azone will adversely affect the partitioning of the drug into the skin.

From the published reports, it would appear that Azone enhances the percutaneous penetration of drugs without detectable toxicity, altering sensitivity or inducing other systemic or local side effects. Radioactive studies have shown that Azone is bound in the epidermis and corium and that only relatively small amounts are released from the skin (68,54). The precise mechanism is unknown but the well documented penetration enhancing effects of Azone could be due to alteration of the lipid-protein structures of the stratum corneum and/or due to its role as a carrier of drug molecules across the skin by formation of ion-pairs.

Collectively, these and other studies reinforce the

concept that design of an optimal vehicle for topical delivery of a drug is likely to involve carefully orchestrated compromises as one attempts to balance often opposing influences of vehicle components with respect to drug solubility, skin partitioning, and its membrane structure altering properties.

Selection of a Model Membrane for *In Vitro* Percutaneous Penetration Studies

A variety of models have been investigated over the years to study percutaneous penetration of drugs in humans. Early work utilizing membraneless systems was conducted by Poulsen *et al.* (69). Ostrenga *et al.* (20,70) studied the release of fluocinolone acetonide and its acetate from propylene glycol-water gel systems into stirred isopropyl myristate acting as a diffusional sink. Various living membranes such as frog skin (71), the membrane encasing the chick embryo (72-74), man-made membranes such as the hydrated dialysis membranes (75), porous cellulosic membranes with lipid-filled interstices (76), and silicone rubber films (77) have also been utilized. Unfortunately, none bears even close resemblance to skin and all of these studies were questionable on the same grounds as membraneless systems

for their ability to mimic *in vivo* conditions.

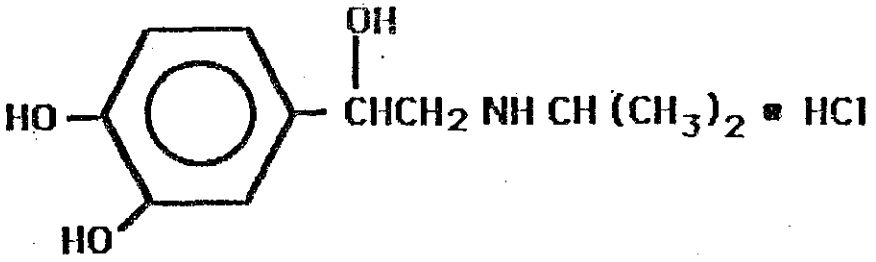
The other alternative to human skin, still popular with some researchers, is the use of animal skin. Wester and Maibach (78), have documented the work of several investigators using animal skin models for percutaneous penetration studies. The skins of various animals such as rats, rabbits, mice, monkeys, and pigs have been used. But, there are documented (79) differences of opinions among the researchers regarding the merits of this approach. Durrheim *et al.* (80) and Stoughton (81) reported that hairless mouse skin is similar to human skin for some compounds, but Bronaugh *et al.* (82), have shown that this may depend on the nature of the compound and is certainly not always the case. Walker *et al.* (83) showed that there was a significant difference in the rates of permeation between the hairless mouse and human skin for various compounds. Similarly, with the rat, Bartek *et al.* (84) concluded that the permeability of the rat skin is not similar to human skin. These apparent discrepancies can be explained by the structural differences between human and animal skin. Although it has been argued (85) that the results of *in vitro* percutaneous penetration studies using human cadaver skin cannot be correlated to *in vivo* percutaneous penetration, human cadaver skin seems to be the most nearly ideal

alternative to human skin in vivo at present. Therefore, in this investigation all penetration studies were conducted using excised human cadaver skin.

Selection of Isoproterenol HCl as a Model Drug

Isoproterenol [4-(1-hydroxy-2[(1-methylethyl) amino]ethyl)-1,2-benzenediol] (Figure 2) is a direct-acting sympathomimetic amine which acts on beta-adrenergic receptors. It is generally used as a bronchodilator and cardiac stimulant. It is readily absorbed when given parenterally or administered as an aerosol. Absorption following oral or sublingual administration is erratic and unreliable (86,87)

Isoproterenol HCl undergoes two types of metabolic transformations after oral administration. It is converted to a sulfate conjugate in the gut wall and it is also metabolized by hepatic catechol-O-methyl transferase to methoxy-isoproterenol (88-90). Following an IV dose, 50% appears unchanged in the urine. After an oral dose, 8% is excreted unchanged and 90% is excreted as the conjugate (91,92). Hence, the oral dose is larger than the parenteral dose. The plasma half-life of isoproterenol hydrochloride ranges from a few minutes after intravenous administration, to 2 h after



4-[1-hydroxy -2- [(1-methylethyl) amino]
ethyl] -1, 2- benzenediol

$C_{11} H_{17} NO_3$ F.W. = 211.24

Figure 2. Chemical structure of isoproterenol HCl.

subcutaneous or sublingual administration and 2-4 h after rectal administration of a sublingual tablet.

Isoproterenol HCl is administered intravenously at a dose of 20-60 mcg initially followed by 10-200 mcg as needed to elicit the needed response (93) indicating significant inter-individual variation. The recommended oral or sublingual dose ranges from 10-15 mg qid, but not more than 60 mg in a day. It is also administered by inhalations as 1:200 - 1:100 solution prn (94). An infusion of 1:500,000 solution is given at a rate of 5 mcg/min (95).

Isoproterenol HCl is highly metabolized when given orally (96). It has a very short biological half-life when administered intravenously, orally or sublingually. Furthermore it shows an erratic and unreliable absorption following oral or sublingual administration. These considerations provided strong incentive for investigating transdermal delivery of isoproterenol HCl. Transdermal delivery of isoproterenol HCl would eliminate first-pass metabolism and the variability in absorption associated with gastrointestinal transit. A successful transdermal system would also increase patient compliance due to a reduction in frequency of dosing associated with intravenous and oral routes.

Preliminary in vitro studies revealed that

isoproterenol HCl, a highly water soluble salt, had a poor penetration rate through human cadaver skin. This was not unexpected in view of its ionic character and in light of the numerous studies (discussed earlier) documenting that percutaneous penetration of most drugs follows Fickian diffusion. In fact, isoproterenol HCl offered an excellent opportunity to test the percutaneous penetration enhancing effects of Azone on an ionizable compound.

Scope of Present Study

This study is intended to examine the effect of various physicochemical factors such as solubility, partition coefficient and pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl from a propylene glycol-water system across excised full-thickness human cadaver skin from the abdominal region. The penetration enhancing effect of Azone on different ionic species of isoproterenol will also be examined and evaluated by varying the vehicle pH. In addition to helping define optimal conditions for transdermal delivery of isoproterenol HCl, it is hoped that this investigation will contribute to a better understanding of the percutaneous penetration of ionizable drugs and lead to

insights into the mechanisms by which Azone enhances the percutaneous penetration of ionizable drugs.

THEORY

Percutaneous penetration of most drugs through human skin is a passive diffusion process described by Fick's first law of diffusion. Equation 1 (97) is the mathematical expression frequently used to describe this membrane-limited transport under steady-state conditions. The amount of drug absorbed through the membrane per unit area per unit time, also known as flux, J can be readily calculated from equation 1, where,

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

D is the diffusion coefficient of the drug within the membrane, h is the membrane thickness and P is the membrane/vehicle partition coefficient. The quantity (D P/h) is referred to as the permeability constant or permeability coefficient, Kp.

$$J = Kp \cdot C \qquad \text{(Eq. 2)}$$

The drug concentration, C, in the vehicle must remain

constant during the course of the penetration experiment and the receptor chamber should mimic a perfect sink. In investigations using human cadaver skin, the stratum corneum generally functions as the rate-limiting barrier. Therefore, the parameters D, P, and h in equation 1 are determined with reference to this skin layer. Upon closer examination, it is evident that equation 1 is very useful in identifying the factors that could be manipulated to control percutaneous penetration rate or flux (J). Assuming that the diffusion coefficient D and skin thickness h remain constant, the flux would be directly related to the product of partition coefficient, P and drug concentration in the vehicle, C. Therefore, flux can be controlled by varying formulation parameters, which alter P and/or C. Both drug partitioning into the skin and drug concentration in the vehicle can be altered by judicious choice of solvents and other adjuvants for a given drug. In practice, one must strike a balance between drug solubility in the vehicle and its partitioning into the skin in order to attain clinically meaningful flux values. To attain this objective, solubility and partition coefficient (98) information of drugs have been utilized to develop predictive models for optimizing drug availability with limited success .

It is well documented (32-36) that the passive

diffusion process across the skin favors neutral molecules or the uncharged form of ionizable molecules due to their lipophilicity. Therefore, the pH of the vehicle plays an important role in the percutaneous penetration of ionizable drugs. The flux of the ionizable drugs can be affected by changes in pH due to resulting alteration in the ratio of charged to uncharged species.

Isoproterenol at any given pH exists as an equilibrium mixture of cationic, anionic, uncharged and zwitterionic species as shown in Figure 3. The concentrations of the various species as a function of pH an shown in Figure 4. These concentrations were calculated on the basis of macro- and micro-dissociation constants (pKa1 = 8.83, pKa2 = 10.19; pK1 = 9.58, pK2 = 8.91, pK3 = 9.44, pK4 = 10.11) at 25°C as published by Sinistri and Villa (99,100). The percentages of the individual species of isoproterenol HCl present at different pH values were calculated by following equations (101-103):

$$\% \text{ Cation} = 100/(1 + pK2/(H+) + pK1/(H+) + pK1.pK3/(H+)^2) \quad \text{(Eq. 3)}$$

$$\% \text{ Uncharged} = 100/(1 + (H+)/pK1 + pK3/(H+) + pK2/pK1) \quad \text{(Eq. 4)}$$

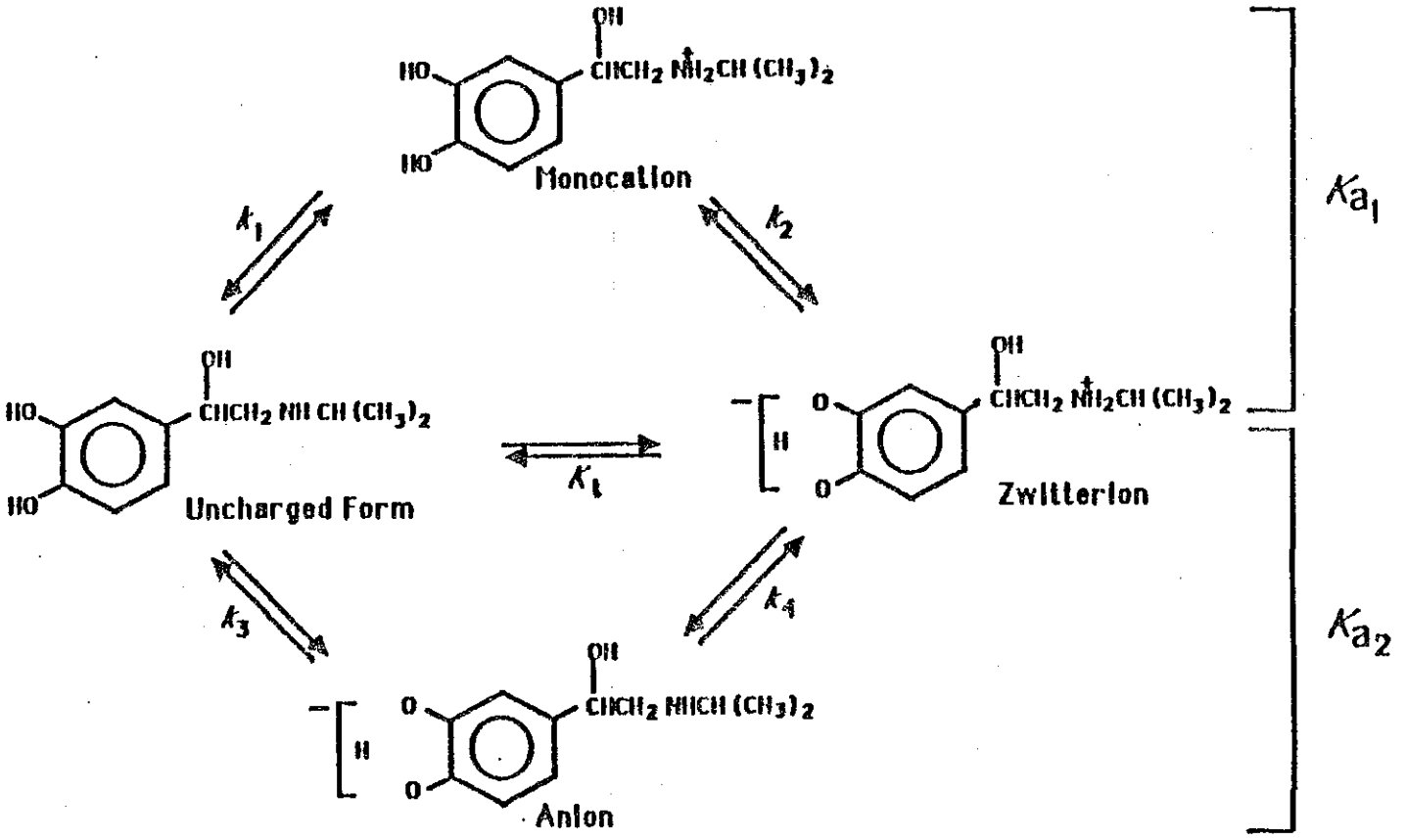


Figure 3. Ionization scheme of isoproterenol HCl.

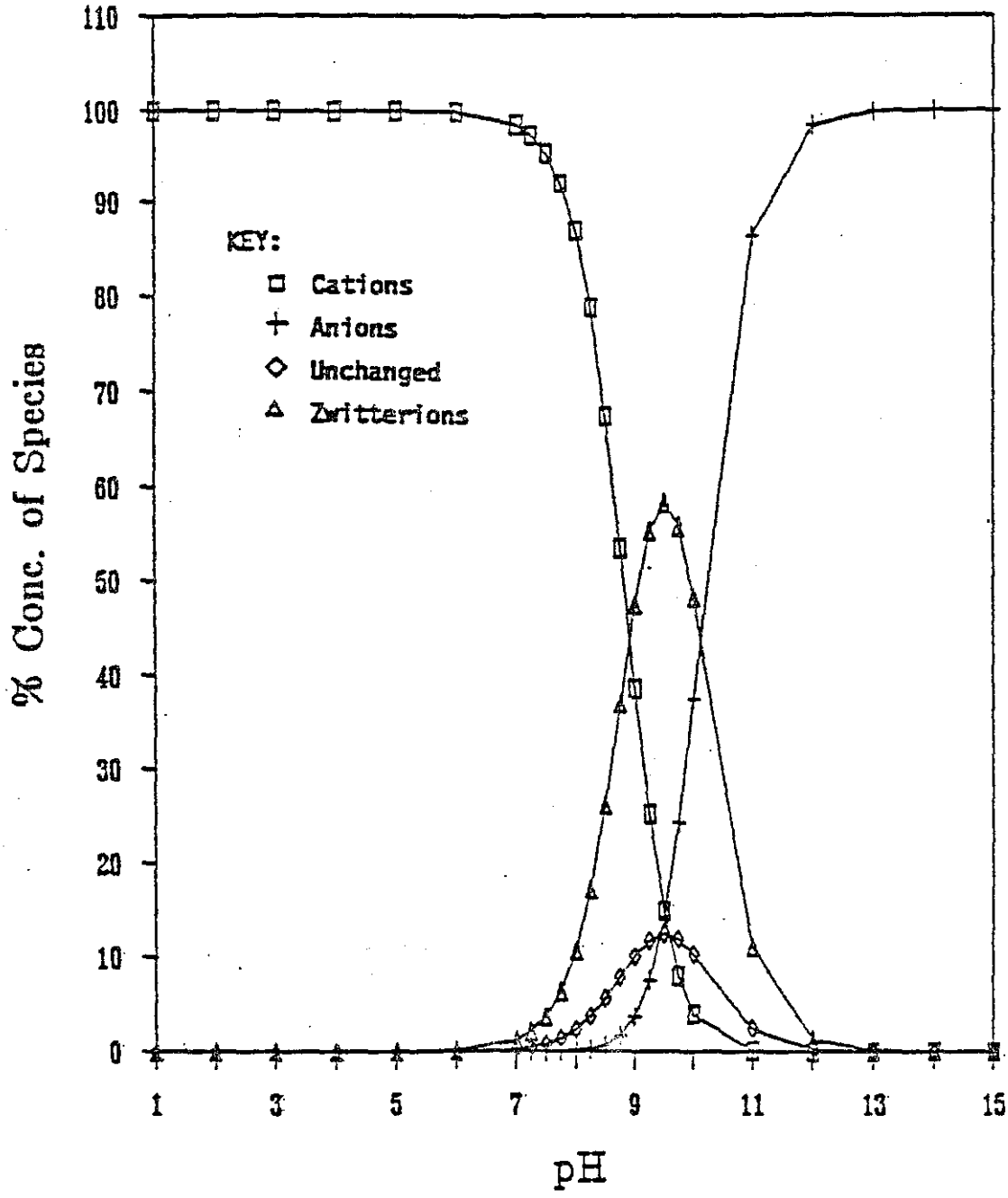


Figure 4. The percent of the different ionic forms of isoproterenol HCl as a function of pH.

$$\% \text{ Zwitterion} = 100/(1 + (H+)/pK2 + pK4/(H+) + pK1/pK2) \quad \text{(Eq. 5)}$$

$$\% \text{ Anion} = 100/(1 + (H+)/pK4 + (H+)/pK3 + (H+)^2/pK1.pK3) \quad \text{(Eq. 6)}$$

Assuming that all species contribute to the total steady-state flux, solely by their own concentration gradient and unaltered by the presence of other species, equation 1 can be rewritten as,

$$J = J^{ca} + J^{an} + J^{n} \quad \text{(Eq. 7)}$$

where the total flux, J is a sum of contributions by cationic (J^{ca}), anionic (J^{an}), and uncharged and zwitterionic (J^{n}) species. By appropriate substitution, we get equation 8:

$$J = (Kp . C)^{ca} + (Kp . C)^{an} + (Kp . C)^{n} \quad \text{(Eq. 8)}$$

The individual permeability coefficients for different species Kp^{ca}, Kp^{an}, and Kp^{n} can then be statistically determined by means of the method of least squares if the flux, J and concentration (C^{ca}, C^{an} and C^{n}) are known. J can be determined experimentally for

any formulation at a given pH and total drug concentration C^{ca}, C^{an} and C^{n} values at any particular pH can be calculated using equations 3, 4, 5 and 6. The calculated permeability coefficients of various species can then be compared to assess the relative permeabilities of human cadaver skin to these species of isoproterenol HCl.

EXPERIMENTAL: PHYSICOCHEMICAL STUDIES

Isoproterenol HCl is known to be very unstable in solution in presence of light, air and in solutions of high pH values. In order to define the experimental conditions and formulation parameters for studying percutaneous penetration of isoproterenol HCl and the penetration enhancing effects of Azone, it was necessary to generate relevant stability, solubility and skin/vehicle partition coefficient data.

The stability of isoproterenol HCl was determined in a series of propylene glycol-water mixtures, normal saline and distilled water. The solubility of isoproterenol HCl in a series of propylene glycol-water mixtures and Azone was also determined. Finally, the partitioning of isoproterenol HCl from various propylene glycol-water mixtures into human cadaver skin was studied to complete this phase of the investigation.

Assay Methods

Analysis of samples for isoproterenol HCl content were carried out by ion-pair high-performance liquid

chromatography by the method of Ghanekar and Das Gupta (104) and also spectrophotometrically (105).

The chromatograph was equipped with a 6000-psi pump[1], a variable wavelength detector[2] and a loop injector[3]. A 250-mm long, 4.6-mm diameter stainless steel column[4] was used. The accurate determination of area under the curve was accomplished by use of an automated integrator system[5]. Injection volume was maintained constant with a 20 ul loop installed in the loop injector and the injections were made with the aid of a 25 microliter gas-tight syringe[6]. The eluent was a 20% (v/v) solution of methanol[7] in water containing 2% (v/v) acetic acid[8] with 0.005 M sodium 1-heptane-

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1. Waters 6000-A pump, Waters Associates, Milford MA.
2. Model 450, Variable Wavelength Detector, Waters Associates, Milford MA.
3. U6K Injector, Waters Associates, Milford MA.
4. Nucleosil C_{18}, Alltech Associates Inc., Deerfield IL.
5. Data Module, Waters Associates, Milford MA.
6. 600 Series syringe, Hamilton Co., Reno NV.
7. HPLC grade, J. T. Baker Chemical Co., Phillipsburg NJ.
8. HPLC grade, J. T. Baker Chemical Co., Phillipsburg NJ.

sulfonate[9]. The pH of the eluent was 2.6 ± 0.05. Epinephrine bitartrate[10] was used as an internal standard. The analysis was conducted at room temperature. The flow rate was 2.0 mL/min at inlet pressure of approximately 1800 psig. The absorbance was measured at 280 nm and the detector was set at 0.1 aufs and sensitivity at 0.02. The chromatogram of a standard mixture of isoproterenol HCl (100 mcg/mL) and epinephrine bitartrate (100 mcg/mL) is shown in Figure 5. The retention time for isoproterenol HCl was 6.25 minutes and for epinephrine bitartrate (internal standard) was 3.91 minutes. A calibration plot constructed using ratio of area under the peak of the standard samples to the internal standard is shown in Appendix 1.

The spectrophotometric analysis of isoproterenol HCl samples was conducted using a Spectronic 710 spectrophotometer[11] set at 280 nm. The standard curves of isoproterenol HCl in distilled water and normal saline are shown in Appendix 2 and 3, respectively.

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9. Lot 26867X, ICN Biomedicals Inc., K & K laboratories, Plainview N.Y.

10. Lot E-4375, Sigma Chemical Co., St. Louis MO.

11. Spectronic 710, Bauch and Lomb, Rochester NY.

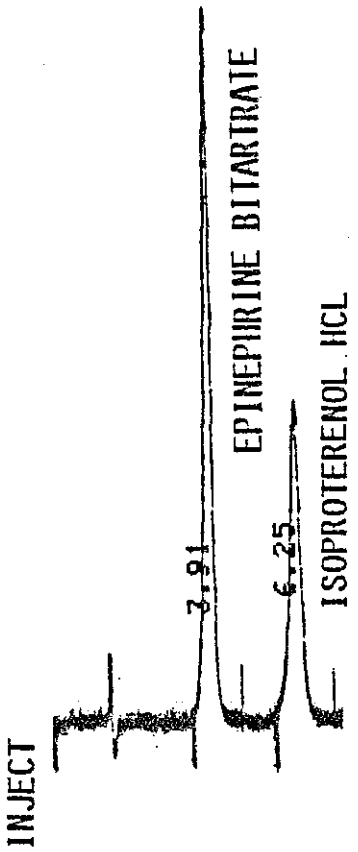

Figure 5. Chromatogram of isoproterenol HCl and epinephrine bitartrate (internal standard).

Column : 10 u Nucleosil C_{18}

Mobile Phase : 20/80 methanol/water with 2% v/v acetic acid and 0.005 M sodium 1-heptane sulfonate, pH = 2.6

Flow Rate : 2.0 mL/min

Chart speed : 0.5 cm/min

Detector : at 280 nm., set at 0.1 AUFS

Stability Studies

Stability in distilled water: The stability of isoproterenol HCl in distilled water was determined in solutions ranging from pH 1.93 to 10.1 at 22° ± 0.5°. Five mL of solutions (100 mg/mL) of isoproterenol HCl buffered[12] to appropriate pH values (1.93-6.53) were stored in glass vials for 24 h. After 24 h one mL of the solution from each vial was withdrawn by a pipette[13] and diluted to 100 mL in a volumetric flask and analyzed by HPLC. The percent isoproterenol remaining was calculated as a mean of three separate determinations. A more detailed study was conducted at pH values 8, 9 and 10 to determine the extent of decomposition after 0, 3, 6, 9, 12 and 24 h periods and the procedure was similar to one described above.

Stability in propylene glycol-water mixture: The stability of isoproterenol HCl[14] was determined in a

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12. pHydrion Buffers, Micro Essential Laboratory Inc., Brooklyn NY.

13. Pipetman (5 mL), West Coast Scientific, Oakland CA.

14. Sigma Chemical Co., St. Louis MO.

series of propylene glycol[15]-water mixtures (10%, 20%, 40% and 60% v/v) at 22° ± 0.5° over a pH range of 1.93-7.38. Five mL samples of freshly prepared solutions (100 mg/mL) of isoproterenol HCl buffered to appropriate pH values in various vehicles were stored in 10 mL glass vials for 24 h. Then one mL of the solution from each vial was withdrawn and pipetted and diluted to 100 mL in a volumetric flask and analyzed by HPLC. The percent isoproterenol remaining was calculated as mean of three separate determinations.

Stability in normal saline: The stability of isoproterenol HCl was determined in normal saline[16] at 22° ± 0.5° and at 37° ± 0.5° in the absence and presence of 0.01% and 0.02% EDTA[17] (ethylenediamine tetraacetic acid), respectively. Freshly prepared solutions (100 mcg/mL) of isoproterenol HCl in normal saline was stored in glass vials at 37° ± 0.5° in an incubator. At time intervals of 1.5, 3, 6, 12 and 24 h samples were taken and analyzed for isoproterenol HCl content by HPLC. The percent isoproterenol HCl remaining was calculated as a

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15. USP grade, J. T. Baker Chemical Co., Phillipsburg NJ.

16. 0.9% Sodium Chloride Injection, USP, Travenol Laboratories Inc., Deerfield IL.

17. Stock # ED2SS, Sigma Chemical Co., St. Louis MO.

mean of three separate determinations.

Solubility Studies:

The solubility of isoproterenol HCl in a series of propylene glycol-water (0, 10, 20, 40, 60 and 100% v/v) mixtures and Azone[18] was determined at 22° ± 0.5°. In all cases, an excess of isoproterenol HCl was added to 5 mL of the solvent in a 50-mL amber colored Erlenmeyer flask with a ground glass stopper. A Teflon-coated magnetic stirring bar was placed in each of the flasks prior to flushing with nitrogen and capping them tightly. The solution was then stirred on a magnetic stirrer for 24 h. All studies were conducted at least in duplicate. Preliminary studies at 24 and 48 h revealed that equilibration could be attained in 24 h. Prior to sampling, the stirring was stopped and the excess drug was allowed to settle. An aliquot of the supernatant was taken and filtered with 0.22u filter using a syringe with millipore adapter[19]. The first few mL of the filtrate were discarded to avoid any error due to the possible saturation and adsorption of the drug to the filter. After appropriate dilution, the concentration was

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18. Lot # 059NO803, Nelson Research, Irvine CA.

19. Swinnex-25, Millipore Filter Corp., Bedford MA.

determined by HPLC as discussed under assay method.

Skin/Vehicle Partitioning Studies

Preliminary experiments had shown that isoproterenol HCl reached equilibrium distribution between the vehicle and skin in three days.

Skin preparations of known weight (100-200 mg) were prepared and equilibrated with solutions of isoproterenol HCl in selected propylene glycol-water mixtures in 50-mL amber colored Erlenmeyer flasks by agitation for 3 days at 22° ± 0.5° after flushing with nitrogen. Initial and equilibrium drug concentrations in the vehicle were assayed using HPLC. The skin samples were washed once with about 5 mL of distilled water and isoproterenol HCl in the skin was determined by homogenizing it with known volume of distilled water. The homogenate was filtered through 0.22u filter paper with a syringe fitted with millipore adapter. The first few drops of the filtrate were discarded due to saturation and adsorption of the drug to the filter. An aliquot of the filtrate was appropriately diluted and analyzed by HPLC.

The skin/vehicle partition coefficient ($K_{S/V}$) was then calculated from equation 11 derived as follows (106):

$$K_{S/V} = \frac{C_S}{C_V} = \frac{M_S/V_S}{M_V/V_V} \qquad \text{(Eq. 9)}$$

where, C_S and C_V are the drug concentrations in the skin and in the aqueous solution, respectively, at equilibrium; M_S and M_V are the masses of isoproterenol in the skin and in the vehicle respectively, at equilibrium; V_S and V_V are the volumes of the skin and the vehicle, respectively. The M_S may be expressed in terms of M_V and M_V^O, the total mass of drug initially present in the aqueous solution:

$$M_S = M_V^O - M_V \qquad \text{(Eq. 10)}$$

Therefore, Eq. 3 may be rearranged to give:

$$K_{S/V} = \frac{(M_V^O - M_V)(V_V)}{M_V (V_S)}$$

$$= \frac{C_V^O - C_V}{C_V} \qquad \text{(Eq. 11)}$$

where C_V^O is the initial concentration of drug in the vehicle.

RESULTS AND DISCUSSION: PHYSICOCHEMICAL STUDIES

Stability Studies

The stability of isoproterenol HCl in distilled water, normal saline, 10%, 20%, 40% and 60% (v/v) propylene glycol in water at 22° ± 0.5° is shown in Table I. The analysis of data revealed that isoproterenol HCl was stable in these vehicles at pH values ranging from 1.9 to 7.38. Almost 99% of the initial amount of isoproterenol HCl present could be accounted for at the end of 24 h. The stability of isoproterenol HCl in distilled water at pH values 8, 9 and 10.1 was also determined in order to study the extent of decomposition under high pH conditions. Table II shows that increasing the pH of the solution resulted in an increase in the rate of decomposition. This information was later utilized to select range of pH values for the percutaneous penetration studies.

Since the fluid in the receptor chamber of the diffusion cell was normal saline maintained at 37° ± 0.5°, it was necessary to study the stability of isoproterenol HCl in normal saline at this temperature.

TABLE I

Stability of Isoproterenol HCl in Water, Normal Saline and Propylene Glycol-Water Mixtures

Vehicle	pH	Amount[1] Left, %	pH	Amount Left, %	pH	Amount Left, %	pH	Amount Left, %
Distilled water	1.9	98.95 ± 0.91	3.8	98.78 ± 4.59	5.2	98.29 ± 2.43	6.5	98.61 ± 1.99
Normal Saline	1.9	98.86 ± 1.30	3.7	96.81 ± 2.04	5.3	98.23 ± 2.04	6.5	99.54 ± 0.59
10% (v/v) PG/DW	2.0	99.24 ± 0.83	3.6	98.7 ± 0.80	5.9	98.72 ± 1.46	6.6	98.75 ± 1.32
20% (v/v) PG/DW	2.0	99.77 ± 0.52	3.8	99.67 ± 0.42	6.3	99.16 ± 0.90	6.7	98.41 ± 1.96
40% (v/v) PG/DW	2.1	99.72 ± 0.06	3.6	98.53 ± 0.83	6.3	101.65 ± 1.09	7.2	98.66 ± 2.19
60% (v/v) PG/DW	2.2	100.24 ± 0.67	3.7	98.28 ± 2.54	7.1	99.92 ± 0.25	7.4	98.85 ± 0.98

[1] Values were determined after storage at room temperature (22 ± 0.5 °C) for 24 hours and are expressed as a mean of three determinations ± standard error of mean.

TABLE II

Stability of Isoproterenol HCl in Distilled Water at pH 8, 9 and 10.1

Time, h	Amount Left, %[1]		
	pH 8.0	pH 9.0	pH 10.1
0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3	99.15 ± 0.00	100.32 ± 0.01	91.62 ± 0.04
6	97.11 ± 0.00	96.60 ± 0.01	60.57 ± 0.02
9	96.10 ± 0.00	88.26 ± 0.02	28.29 ± 0.17
12	98.03 ± 0.00	73.31 ± 0.01	36.57 ± 0.02
24	94.07 ± 0.01	50.81 ± 0.02	30.61 ± 0.01

[1] Values are determined at 22 ± 0.5 °C and expressed as mean of three determinations ± standard error of mean.

The stability of isoproterenol HCl in normal saline with and without 0.01% and 0.02% EDTA was determined at 37° ± 0.5° (Table III). The traces of metallic ionic impurities in aqueous solutions of isoproterenol HCl are reported to accelerate its oxidative decomposition. EDTA has been used alone and in conjunction with other antioxidants to stabilize the aqueous formulations of isoproterenol HCl (107). In the present study, 0.01 and 0.02 % concentrations of EDTA did not produce marked improvement in stability of isoproterenol HCl in normal saline. Therefore, EDTA was not used to stabilize isoproterenol HCl solutions in this study. The data in Table III also revealed that decomposition of the drug in normal saline was minimal during first 2 h. Therefore sampling times in percutaneous penetration studies never exceeded 2 h.

Solubility Studies

The solubilities of isoproterenol HCl in distilled water, propylene glycol, Azone and 10%, 20%, 40% and 60% (v/v) propylene glycol in water at 22° ± 0.5° are shown in Table IV, Figure 6.

Isoproterenol HCl is highly soluble in water, moderately soluble in propylene glycol and poorly soluble

TABLE III

Stability of Isoproterenol HCl in Normal Saline at 37 ± 0.5 °C

Time, h	Amount Left, %[1]		
	Normal Saline	Normal Saline + 0.01% EDTA	Normal Saline + 0.02% EDTA
0.0	100.00 ± 0.00	100.00 ± 0.11	100.00 ± 0.11
1.5	100.21 ± 0.14	96.38 ± 0.58	102.38 ± 0.16
3.0	88.5 ± 0.06	84.59 ± 0.05	92.59 ± 0.10
6.0	88.26 ± 0.11	91.08 ± 0.18	98.19 ± 0.19
12.0	81.52 ± 0.07	79.67 ± 0.07	85.93 ± 0.25
24.0	50.33 ± 0.08	60.1 ± 0.13	61.73 ± 0.16

[1] Values are expressed as mean of three determinations ± standard error of mean

TABLE IV

Solubility of Isoproterenol HCl in Selected Solvents

Vehicle[1]	N	pH[2,3]	Solubility, mg/ml[3,4]
Distilled Water	6	3.9 ± 0.1	388.9 ± 8.5
10% PG/DW	2	3.9 ± 0.3	357.8 ± 5.6
20% PG/DW	2	4.0 ± 0.1	335.1 ± 10.3
40% PG/DW	2	4.1 ± 0.3	288.3 ± 2.2
60% PG/DW	2	4.3 ± 0.4	243.3 ± 2.0
100% PG	6	3.9 ± 0.1	91.6 ± 3.4
Azone	3		8.7 ± 0.1

1 All percentages are expressed as v/v.

2 All solubility and pH values are expressed as mean + standard error of mean.

3 Determination using a Corning Model 125 pH meter with glass electrode Ag/AgCl internal reference electrode.

4 The solubilities were determined at 22 ± 0.5 °C.

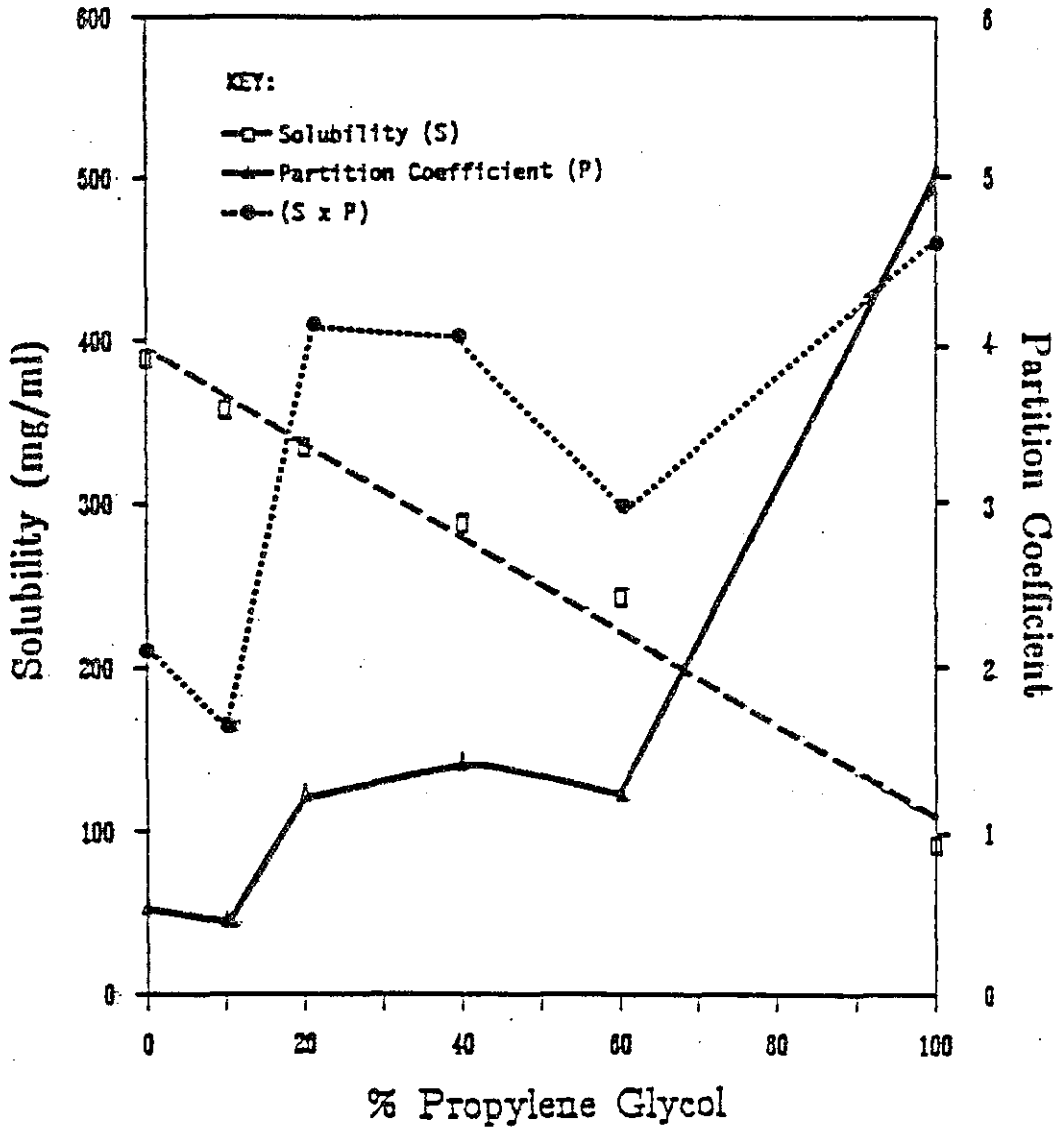

Figure 6. Plot of solubility (S), partition coefficient (P) and (P x S) vs percent propylene glycol.

in Azone. The decrease in the solubility of isoproterenol HCl with increasing percentages of propylene glycol was as predicted by the following relationship:

$$\text{solubility} = -2.88 \ \% \text{ propylene glycol} + 394.39 \quad \text{(Eq. 12)}$$

Skin/Vehicle Partitioning Studies

The skin/vehicle partition coefficient values of isoproterenol HCl are shown in Table V, Figure 6. The various vehicles studied were distilled water, propylene glycol and 10%, 20%, 40% and 60% v/v propylene glycol in water.

The partition coefficient was found to increase as percentage of propylene glycol in the vehicle was increased indicating the increasing tendency of drug molecules to partition into the skin from systems rich in propylene glycol.

Selection of Optimal Vehicle

Equation 1 predicts that the penetration rate across the stratum corneum is directly proportional to the

TABLE V

Solubility and Skin/Vehicle Partition Coefficent of Isoproterenol HCl in Propylene Glycol/Water Mixtures

Vehicle[1]	Solubility (S), mg/ml[2,3]	Partition Coefficient (P)[2,3]	S x P
Distilled Water	389.0 ± 8.5	0.546 ± 0.083	212.5
10% PG/DW	357.8 ± 5.6	0.465 ± 0.067	166.5
20% PG/DW	335.1 ± 10.3	1.247 ± 0.067	418.1
40% PG/DW	288.3 ± 2.2	1.438 ± 1.314	414.4
60% PG/DW	243.3 ± 2.0	1.527 ± 0.353	371.6
Propylene Glycol	91.6 ± 3.4	5.066 ± 1.341	464.1

1 All percentages are expresses as v/v.

2 The tabulated values are expressed as mean ± standard error of mean.

3 Determined at 22 ± 0.5 °.

product of the partition coefficient and drug concentration present in the donor phase if skin thickness (h) and diffusion coefficient of the drug in the skin are kept constant. As skin/vehicle partition coefficient and solubility are dependent on the composition of the vehicle, these parameters could be manipulated to attain a desired penetration rate by judicious selection of an appropriate vehicle.

Figure 6 represents the relationship of solubility, S and partition coefficient, P and their product, P.S, as a function of percent propylene glycol in the vehicle. It is seen from this plot that as the percent propylene glycol increased in the vehicle, the solubility of isoproterenol HCl decreased. On the other hand, there was an increase in the partitioning of the drug into the skin. The product P.S dropped initially and then increased dramatically as the propylene glycol concentration in water reached 20% (v/v). Further increases in propylene glycol concentration did not show significant improvement in P.S values, although the maximum P.S value was recorded for 100% (v/v) propylene glycol. Therefore, one could predict that 100% (v/v) propylene glycol in the water would provide maximum flux of isoproterenol HCl across the human skin. However, previous studies have established that percentages higher

than 40% (v/v) of propylene glycol in the vehicle cause dehydration of skin and produce irritational effects. In view of these considerations 20% (v/v) propylene glycol in water would appear to be the optimal vehicle for development of a transdermal delivery system for isoproterenol HCl.

EXPERIMENTAL: IN VITRO STUDIES

This phase of the current investigation included the study of the effect of vehicle composition and penetration enhancer, Azone, on percutaneous penetration of isoproterenol HCl. Further, studies were also conducted to understand the relative contributions of various species of isoproterenol HCl to the observed flux at any given pH. The buffered formulations containing specific concentrations of the drug at specific pH values were applied to human cadaver skin and the flux was calculated from the collected penetration data. The permeability coefficients of various species of isoproterenol HCl were then calculated by the procedure discussed earlier in the section on theory, and then compared to determine the relative permeability of human cadaver skin to these species. The same buffered formulations were also examined through Azone-pretreated skin at specific pH in order to determine if the effect of Azone on the skin differentiated between cationic, anionic and neutral forms of isoproterenol HCl.

Preparation of skin: All of the penetration

experiments reported here utilized human abdominal skin obtained at autopsy. Immediately following excision, the skin was placed in a plastic bag and stored in a freezer for periods up to (but not exceeding) three months. This method of storage has been reported not to damage the skin (108,109). Before the experiment, the skin was allowed to thaw gradually to room temperature and the skin was placed on a smooth dissection board with the epidermal surface flat and in contact with the board. Subcutaneous fat was completely removed using a scalpel and 6-10 pieces of suitable sizes were cut from each specimen representing skin of a single donor.

Experimental Design for Skin Diffusion Studies: Each piece of skin was mounted in a special glass diffusion cell as illustrated in Figure 7. The skin cell consisted of a lower chamber with a sampling port. A Teflon-coated magnetic bar was placed at the bottom of the cell to provide efficient mixing. The lower chamber was enclosed by a water jacket which allowed circulation of water at the selected temperature. The skin was placed in position on an O-ring between two ball joints of the top and bottom chambers, using a pitch type, ground-joint clamp. The diffusion area was 2.01 cm^2. The epidermal side of the skin was covered with a loosely

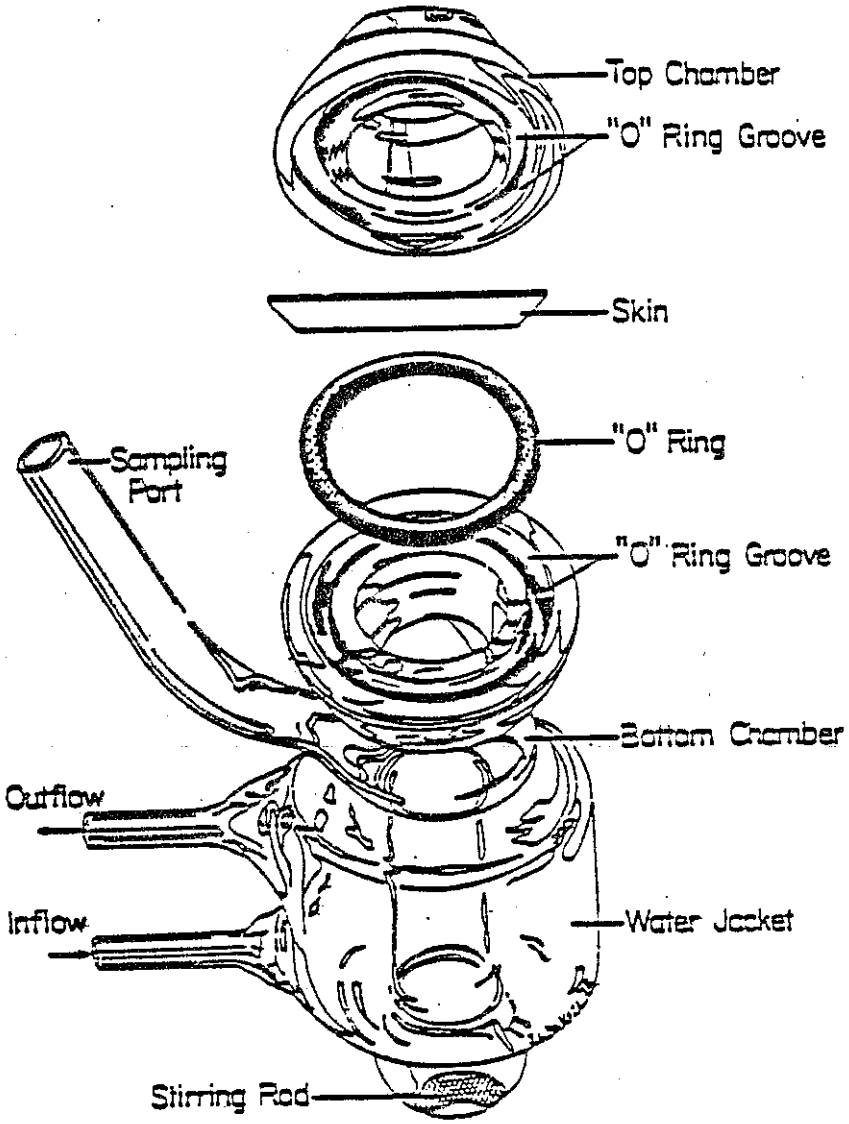

Figure 7. Diagrammatic representation of the diffusion cell used in penetration studies. Reproduced from BenKorah (65).

fitting rubber stopper to prevent moisture loss. Normal saline was pipetted into the skin cell bathing the dermal side. The sampling port was covered by a plastic film[20] and any air bubbles on the dermal side were carefully removed by slightly tilting the cell back and forth. Each cell was mounted on a magnetic motor. The temperature of the fluid in the receptor chamber was maintained at 37° ± 0.5° by circulating water from a constant temperature water circulator[21] through the jacket of each cell in order to stimulate in vivo temperature conditions. After mounting, each piece of skin was allowed to stand for 12 h before beginning the experiment in order to equilibrate with respect to the temperature and relative humidity of the environment.

One-and-a-half mL of freshly prepared solution of isoproterenol HCl in appropriate vehicle was pipetted onto the epidermal side of the skin, the rubber stopper replaced and the entire skin cell assembly wrapped with aluminum foil to protect it from light.

All experiments were carried out for periods of either 12 or 24 h. At selected time intervals (1, 2, 4, 6, 8, 10, 12 and 24 h) the receptor solution from the

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20. Nugget^{R}, All-Purpose Film, Nugget Distributor, Stockton CA.

21. Haake, Model-FE, VWR Scientific Inc., San Francisco CA.

bottom chamber was completely removed through the sampling port using a disposable syringe with its needle attached to a thin flexible plastic tubing. This allowed for a quick and complete removal of the receptor solution and refilling with fresh normal saline solution. The receptor-fluid samples withdrawn were immediately assayed for isoproterenol HCl content spectrophotometrically. Random samples were also assayed by high pressure liquid chromatography and the calculated amount penetrated by both methods were compared to check on any unexpected decomposition of the drug during penetration studies.

Effect of Propylene Glycol: One-and-a-half mL of freshly prepared suspensions of isoproterenol HCl in 0%, 20%, 60% and 100% (v/v) propylene glycol in water were applied to the skin in order to determine the effect of propylene glycol on percutaneous penetration of isoproterenol HCl. The thermodynamic activity of the drug in the respective solvent systems was maintained constant by incorporating 10% excess drug over its solubility.

Effect of Azone: The effect of the penetration enhancer, Azone, on the penetration of isoproterenol HCl through human cadaver skin was studied by incorporating selected concentrations of Azone in the formulations and by

pretreating the skin with Azone.

The effect of Azone concentration on percutaneous penetration of isoproterenol HCl was studied from suspensions of Azone in 20% (v/v) propylene glycol in water and from gels made with 4% (w/w) hydroxypropyl cellulose[22] in 20% (v/v) propylene glycol in water. The suspensions of 1%, 5%, and 10% (v/v) of Azone in 20% (v/v) propylene glycol in water were made by agitating a precalculated amount of Azone in 20% (v/v) propylene glycol/water mixture and then adding an excess drug to it. The amount of drug in excess of solubility ensured constant concentration gradient during the penetration experiment. The suspensions containing 1%, 5% and 10% (v/v) Azone in 20% (v/v) propylene glycol/water mixture were gelled with 4% (w/w) hydroxypropyl cellulose and the calculated amount of drug was added to ensure constant drug concentration in solution during the experiment. The penetration study was conducted by applying 1.5 mL of these gels to the epidermal surface of the skin.

In the pretreatment studies, the human cadaver skin piece mounted on the diffusion cell was pretreated with 0.1 mL of neat Azone for periods of 3, 30, 60 and 720

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22. Klucel^{R}, Grade HF, Lot # 3027, Hercules Inc., Wilmington DE.

minutes. The epidermal side of the skin was rinsed first with 100% ethanol and then with distilled water. It was then wiped clean with a micro-wipe[23]. Penetration studies were conducted after applying 1.5 mL of freshly prepared suspensions of isoproterenol HCl in 20% (v/v) propylene glycol-water maintaining the thermodynamic activity constant as before.

Effect of the pH of the Vehicle on Human Cadaver Skin: Twelve diffusion cells were prepared from two separate pieces of skin. These cells were divided into three groups of 4 cells each. The mean flux from suspensions of isoproterenol HCl in distilled water was determined for each group for the 12-h study. Then, the donor chamber was washed five times with 1 mL distilled water each time. The receptor chamber was emptied, rinsed and refilled with fresh normal saline, and the donor chambers were refilled with appropriate solution. Group 1 was treated with distilled water (control), group 2 was treated with buffered solution of pH 2, and group 3 was treated with buffered solution of pH 10 for 12 h. Then, again the donor chambers were washed with distilled water (5 x 1 mL). The receptor chamber was emptied and filled with fresh normal saline, the donor chamber was covered

23. Scott Paper Co., Philadelphia PA.

with parafilm and the cells were allowed to equilibrate for 12 h. The percutaneous penetration study was conducted again from suspensions of isoproterenol HCl in distilled water for 12 h. The mean flux values calculated for each group were compared with flux values for the same skin sample prior to exposure to respective pH buffer. The data were analyzed statistically by ANOVA. This experimental design was dictated by size of the available skin samples from human abdominal region and requirement of 10-12 skin pieces from each available skin sample.

Effect of pH on Percutaneous Penetration of Isoproterenol HCl: Considerable biological variation exists among different skin specimens with regard to percutaneous penetration (110). The effect of this variable was minimized by utilizing skin specimens from the same regional site for each experiment. Five sets of penetration experiments were carried out in order to have statistically meaningful results. In each set, 10 diffusion cells were prepared using abdominal skin samples from the same donor - two for each pH. This limitation was dictated by the size of the skin samples available from the same site.

The percutaneous penetration study was conducted

using buffered solutions of pH values ranging from 1.5 to 9.5 containing known amounts of isoproterenol HCl. The mean flux values were calculated after 12-h study. Then the donor chamber was washed with distilled water (5 x 1 mL) and the receptor chamber was rinsed with normal saline. After refilling the receptor chamber with fresh normal saline, the cells were equilibrated for 12 h. The epidermal side was exposed to pure Azone for an hour, washed with ethanol (5 x 1 mL) and then distilled water (5 x 1 mL) to remove excess Azone. The donor side was then refilled with the same buffered formulations (but freshly prepared) and the penetration study was repeated. Permeability coefficients for normal and Azone-pretreated skin samples were calculated from experimental flux and concentration values using the method of least squares.

RESULTS AND DISCUSSION: IN VITRO STUDIES

Effect of Propylene Glycol

All of the penetration data from suspensions of isoproterenol HCl (10% in excess) in various propylene glycol-water mixtures was analyzed by plotting Q (amount penetrated per unit area) against time. A regression analyses of the steady-state region of the penetration curves (Figure 8) were carried out using a computer[24] and the data are presented in Table VI.

Figure 9 shows that as the percentage of propylene glycol in the vehicle increased, the flux increased initially and then remained essentially constant for vehicles containing 20 to 100% (v/v) propylene glycol. It is instructive to note that this curve parallels the P.S plot shown in Figure 6. It would appear that increased partitioning of isoproterenol HCl with increasing propylene glycol concentrations and the previously reported (25,26) decrease in the diffusional resistance of the skin by propylene glycol did not adequately compensate for decreased thermodynamic flux.

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24. TeleVideo TPC II/D, Sunnyvale CA.

TABLE VI

Effect of Propylene Glycol on *In Vitro* Percutaneous Penetration of Isoproterenol HCl.

Propylene Glycol, % v/v	Solubility, mg/ml	Flux (J)[1], $mcg/cm^2/h$	Correlation Coefficient, r	Permeability Coefficient (Kp)[2], $cm/h \times 10^{-3}$
0	389.0	14.70 ± 1.01	0.9988	0.378
20	335.2	21.56 ± 0.78	0.9997	0.643
60	243.3	21.59 ± 2.55	0.9979	0.887
100	91.6	19.70 ± 1.91	0.9976	2.150

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Calculated from $J = Kp\ C$

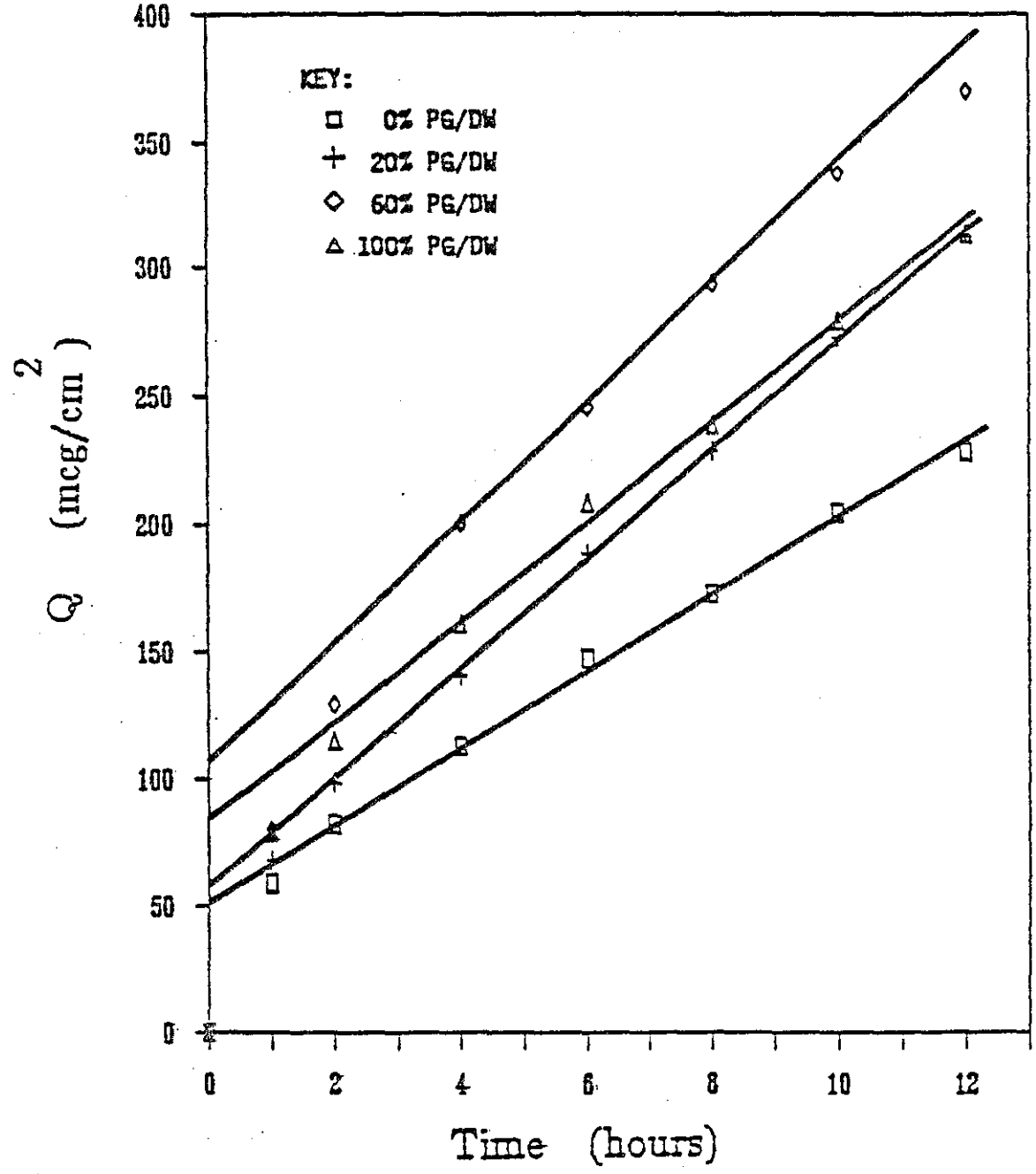

Figure 8. Effect of propylene glycol on <u>in vitro</u> percutaneous penetration of isoproterenol HCl.

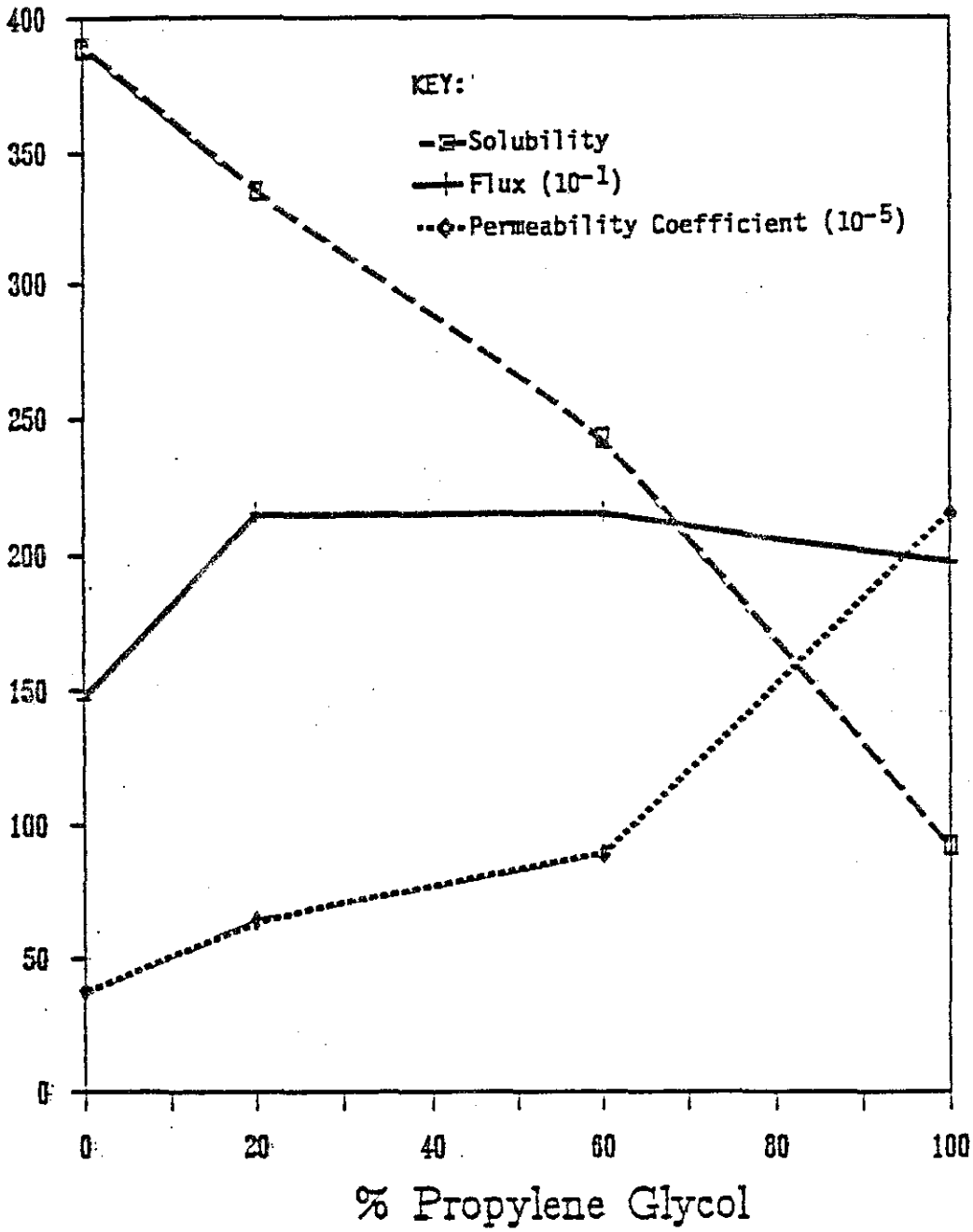

Figure 9. Effect of propylene glycol on solubility, flux and permeability coefficient of isoproterenol HCl.

The important role of propylene glycol as a solubilizer for sparingly soluble non-ionizable drugs in dermatological vehicles has been documented by several early investigators (17-20). Although increased solubility with increasing propylene glycol content in the system was accompanied by decreasing the skin/vehicle partition coefficient, it had been possible to attain desired flux rates for sparingly soluble non-ionizable drugs by carefully balancing the two parameters. Recent work of Polano and Ponec (111) and Portnoy (112) lends additional support to the early work. In this context, present data on percutaneous penetration of an ionizable, highly water-soluble drug, isoproterenol HCl are interesting since drug solubility decreases while partitioning into the skin increases with increasing propylene glycol concentrations. Regardless, these data lend credibility to Fickian diffusion of drugs through human skin, stressing the importance of a balance between solubility and skin/vehicle partitioning of the drug. More importantly, these results suggest the selection of 20% (v/v) propylene glycol-water as the preferred vehicle for topical formulation of isoproterenol HCl as predicted earlier on the basis of solubility-partition coefficient considerations.

Effect of Azone

Comparison of flux values obtained from suspensions of 1, 5 and 10% (v/v) Azone and excess drug in 20% (v/v) propylene glycol-water (Table VII, Figure 10) revealed no statistically significant differences although 1% (v/v) Azone appeared to show the maximum effect. On the other hand, when the percutaneous study was conducted after gelling the suspension formulations listed in Table VII with 4% (w/w) hydroxypropyl cellulose, rather interesting effects were observed. In the absence of Azone, gelling the suspension decreased the flux as revealed by comparison of fluxes for control-1 and control-2 (Table VIII, Figure 11). Incorporation of Azone in the gel formulation more than compensated for increased diffusional resistance of gel resulting in overall increase in flux. As previously observed with suspension formulation, 1% (v/v) Azone was most effective.

In order to examine whether the observed enhancement of drug penetration by Azone was due to lowered skin resistance to drug diffusion, another study was conducted. In this study the skin was pretreated with Azone for periods of 3, 30, 60, and 720 minutes, prior to application of a suspension of isoproterenol HCl in 20% (v/v) propylene glycol in water. The mean steady-state

TABLE VII

Effect of Azone on *In Vitro* Percutaneous Penetration of Isoproterenol HCl from Suspensions.

Azone Concentration, % v/v	Flux (J),[1] (mcg/cm^2/h)	Correlation Coefficient, r	Test For Parallelism
0[2]	10.68 ± 1.76	0.9931	-
1[3]	12.00 ± 1.47	0.9962	P > 0.2
5[3]	8.12 ± 1.94	0.9969	P > 0.2
10[3]	9.07 ± 1.74	0.9906	P > 0.2

1 The tabulated flux values are reported ± 95% confidence limits.

2 Control was a suspension of isoproterenol HCl in 20/80 PG/water without Azone.

3 Test formulations were suspensions of Azone in 20/80 PG/water with drug 10% in excess of solubility.

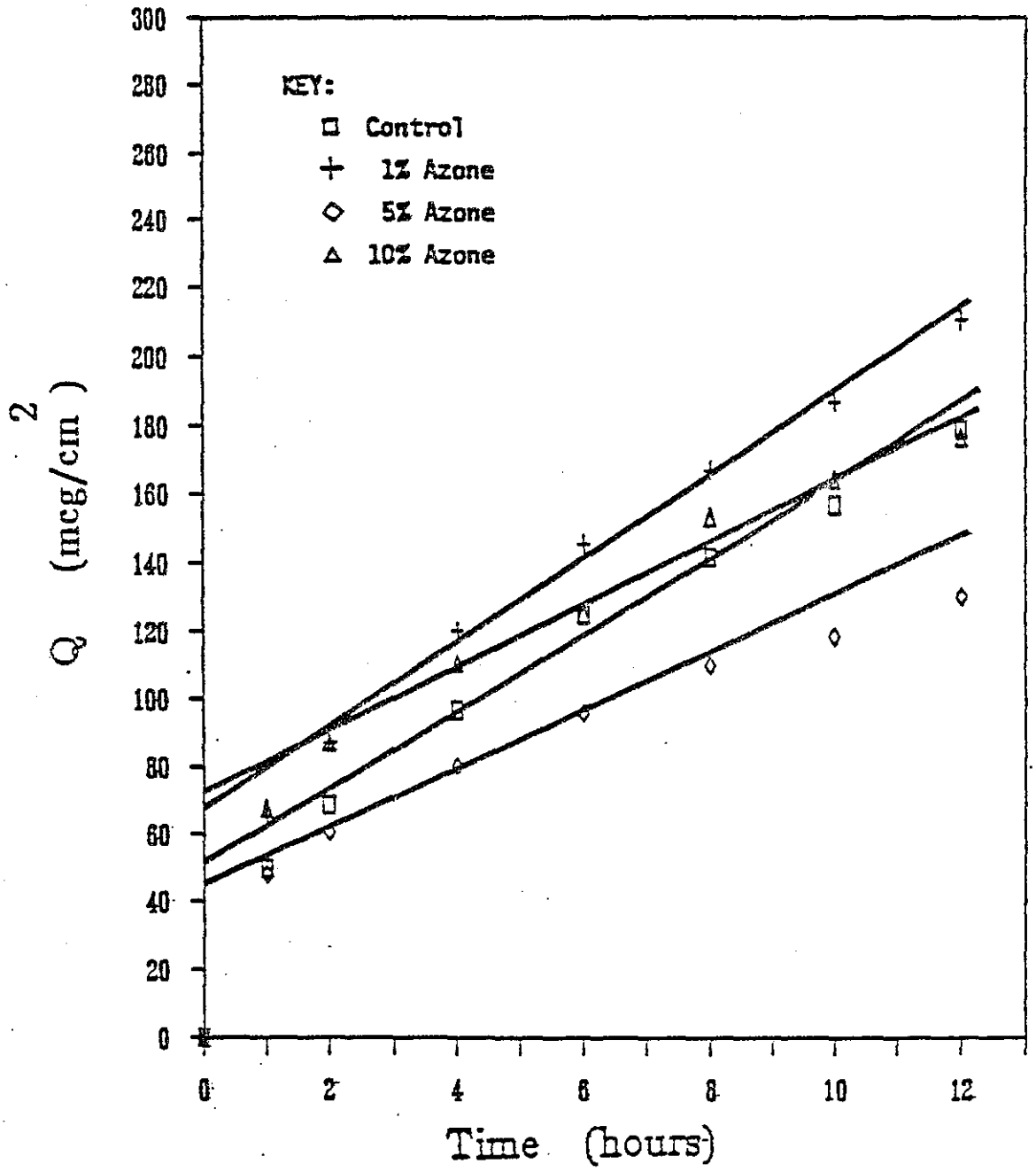

Figure 10. Effect of Azone on in vitro percutaneous penetration of isoproterenol HCl from suspensions.

TABLE VIII

Effect of Azone on *In Vitro* Percutaneous Penetration of Isoproterenol HCl from Gels.

Azone Concentration, % v/v	Flux (J),[1] mcg/cm^2/h	Correlation Coefficient, r	Test For Parallelism Control-1	Test For Parallelism Control-2
Control-1[2]	42.93 ± 6.56	0.9913	-	-
Control-2[3]	13.64 ± 1.84	0.9932	-	-
1[4]	104.16 ± 4.38	0.9991	$P < 0.01$	$P < 0.01$
5[4]	95.11 ± 4.44	0.9992	$P < 0.025$	$P < 0.025$
10[4]	86.69 ± 10.29	0.9930	$P < 0.05$	$P < 0.05$

1 The tabulated flux values are reported ± 95% confidence limits.

2 Suspension of isoproterenol HCl in 20/80 PG/water without Azone.

3 Suspension isoproterenol HCl in 20/80 PG/water gelled with 4% (w/w) hydroxypropyl cellulose.

4 Control-2 formulation with 1%, 5% and 10% (v/v) Azone, respectively.

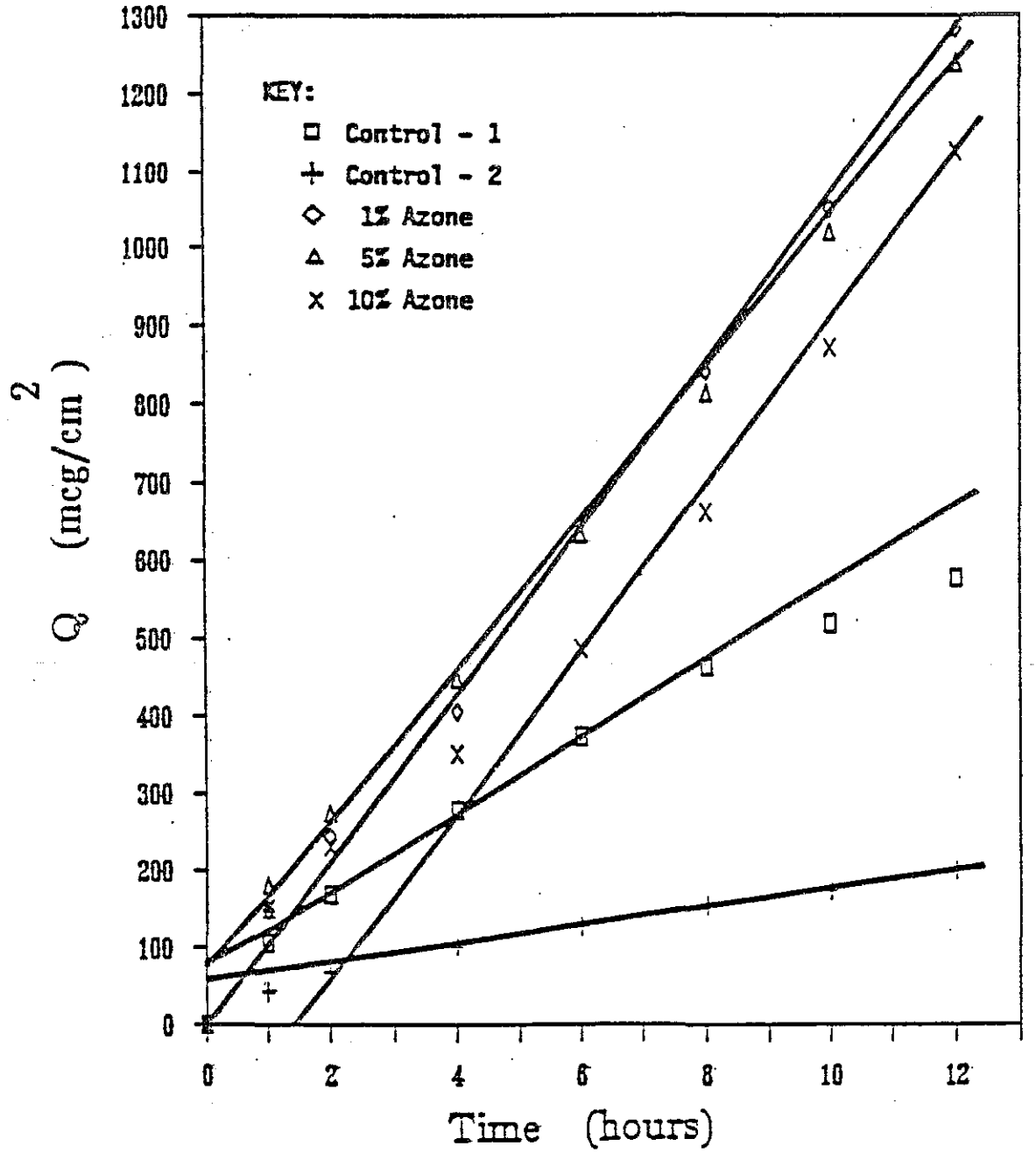

Figure 11. Effect of Azone on in vitro percutaneous penetration of isoproterenol HCl from gels.

flux values for each treatment period were compared with that of untreated control skin from same donor. The results, presented in Figure 12 and Table IX, revealed that the fluxes of 30- and 60-minute pretreated samples were significantly different ($P < 0.025$) from the untreated control. Single 30-60 minute exposure to Azone provided maximum rate of percutaneous penetration which is in agreement with the work of BenKorah et al. (65) on benzocaine. Therefore, 60-minute pretreatment period was selected for subsequent studies.

In order to test these findings further, yet another experiment was conducted using skin from abdominal region of the same donor. A suspension of excess isoproterenol HCl in 20% (v/v) propylene glycol-water was applied to 60-minute Azone-pretreated skin and untreated skin. A similar formulation with 1% (v/v) Azone was applied to another untreated skin in the same study. An analysis of the mean steady-state flux values presented in Table X (Figure 13) showed that pretreatment of skin was more effective in enhancing penetration of isoproterenol HCl through human cadaver skin than the incorporation of Azone in the formulation, strongly suggesting direct effect on the barrier characteristics of skin.

TABLE IX

Effect of Azone Pretreatment on *In Vitro* Percutaneous Penetration of Isoproterenol HCl

Pretreatment Time, minutes	Flux (J),[1] mcg/cm^2/h	Correlation Coefficient, r	Test For Parallelism
Control[2]	12.97 ± 3.78	0.9877	-
3	12.22 ± 2.25	0.9913	$P > 0.35$
30	23.16 ± 3.94	0.9984	$P < 0.025$
60	23.85 ± 0.89	0.9999	$P < 0.025$
720	19.13 ± 1.12	0.9987	$P < 0.01$

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Untreated skin.

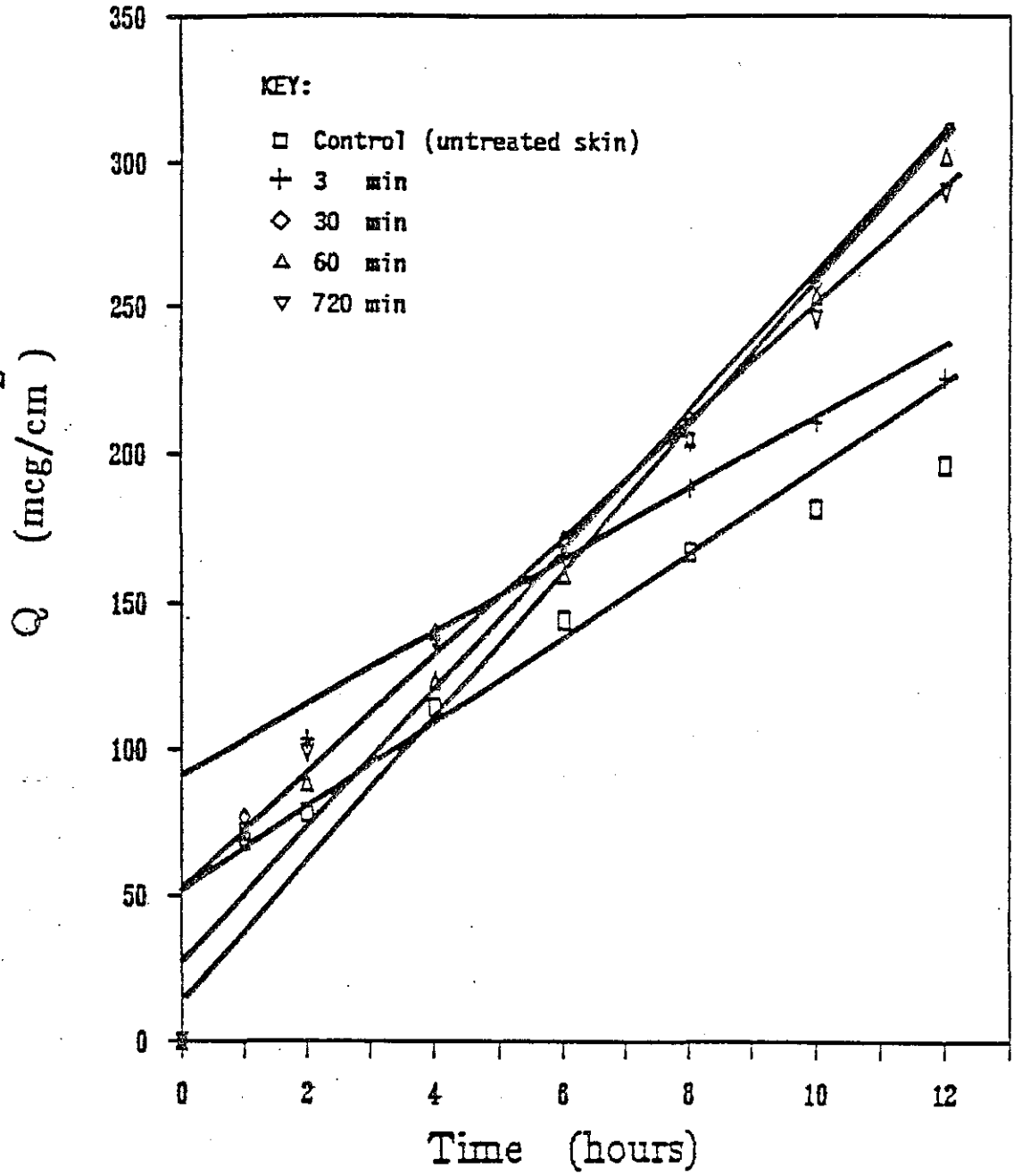

Figure 12. Effect of Azone pretreatment on in vitro percutaneous penetration of isoproterenol HCl.

TABLE X

Comparison of the Effect of 1% (v/v) Azone in the Vehicle and Azone Pretreatment (60 minutes) on *In Vitro* Percutaneous Penetration of Isoproterenol HCl

	Flux (J),[1] mcg/cm^2/h	Correlation Coefficient, r	Test For Parallelism
Control[2]	12.97 ± 3.78	0.9877	-
Pretreatment[3]	23.85 ± 0.89	0.9999	$P < 0.025$
1% (v/v) Azone[4]	16.15 ± 2.41	0.9943	$P < 0.05$

1 The tabulated flux values are reported ± 95% confidence limits.

2 Skin exposed to suspension of isoproterenol HCl in 20/80 PG/Water without Azone.

3 Skin pretreated with pure Azone for 60 minutes and exposed to suspension of isoproterenol HCl in 20/80 PG/water.

4 Skin exposed to suspension of isoproterenol HCl in 20/80 PG/Water with 1% (v/v) Azone

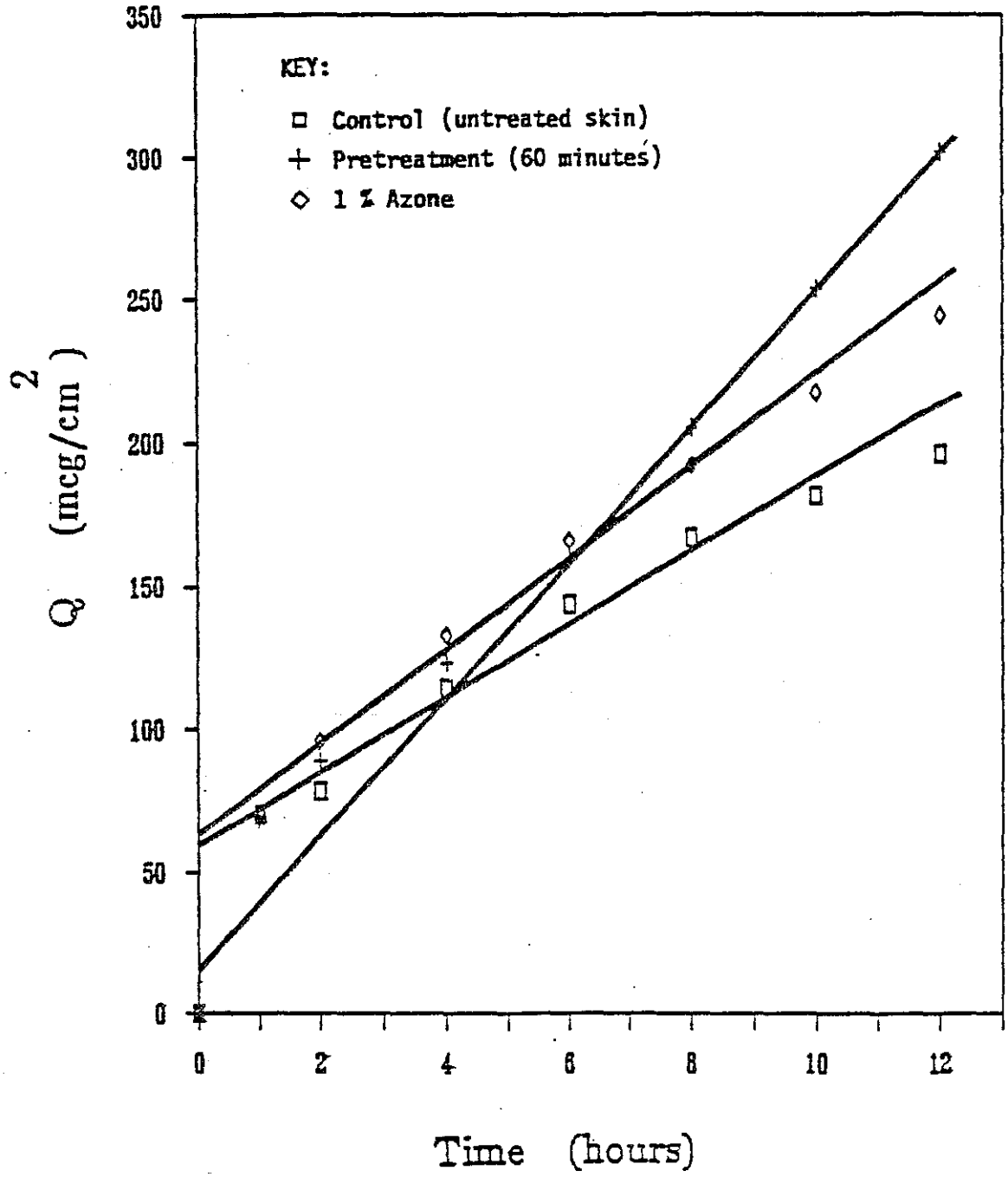

Figure 13. Effect of 1% Azone in the vehicle and Azone pretreatment (60 minutes) on in vitro percutaneous penetration of isoproterenol HCl.

Effect of pH of the Vehicle on Human Cadaver Skin

The effect of hydrogen ion concentration per se and the effect of Azone on percutaneous penetration of isoproterenol HCl were examined over a pH range of 1.5 to 9.5. The experimental design dictated by the size of the available skin specimen from the same region of the same donor required reusing the skin. In order to relate changes in flux at various pH levels to effects of Azone, it was necessary to account for any changes in the permeability of skin caused by deterioration with time and/or exposure to extreme pH conditions.

A percutaneous penetration study was done by treating the skin with buffered solutions of pH 2 and pH 10. The control in the study was treated with distilled water. Table XI and figure 14 show the in vitro effects of pH on the skin. Comparing the flux values of isoproterenol HCl through the skin before and after treatment of the skin sample with a solution of respective pH, it is seen that there was no significant difference ($P > 0.05$) in the flux values as checked by testing the parallelism of the steady-state regions of the two curves, although, there was significant difference ($P < 0.05$) in the amount penetrated per time interval as tested by paired T-Test. The presence of a

TABLE XI

Effect of pH of the Vehicle on Human Cadaver Skin

Time, h	Q (mcg/cm^2)					
	Group I		Group 2		Group 3	
	Untreated	Treated, pH 6.9	Untreated	Treated, pH 2	Untreated	Treated, pH 10
0	0.00	0.00	0.00	0.00	0.00	0.00
1	77.20	23.15	88.68	12.00	67.74	13.64
2	168.24	75.27	134.80	20.93	91.17	21.22
4	321.11	168.90	190.62	43.02	114.71	30.24
6	491.57	320.19	259.22	94.90	135.26	53.10
8	682.79	470.47	340.89	161.87	155.24	75.67
10	849.21	628.68	435.72	238.64	176.85	100.55
12	1020.25	818.16	523.16	321.65	190.00	129.18
Flux (J), $mcg/cm^2/h$	86.22	80.35	39.41	35.05	10.83	12.27
95% C.L.	± 3.22	± 7.20	± 4.97	± 5.65	± 1.58	± 1.14
Correlation Coefficient, (r)	0.9996	0.9988	0.9959	0.9962	0.9921	0.9987
Test for Parallelism	P > 0.05		P > 0.05		P > 0.1	
Paired T-Test[1]	P < 0.001		P < 0.001		P < 0.001	

[1]Paired t-test was applied on mean amount penetrated at each time interval.

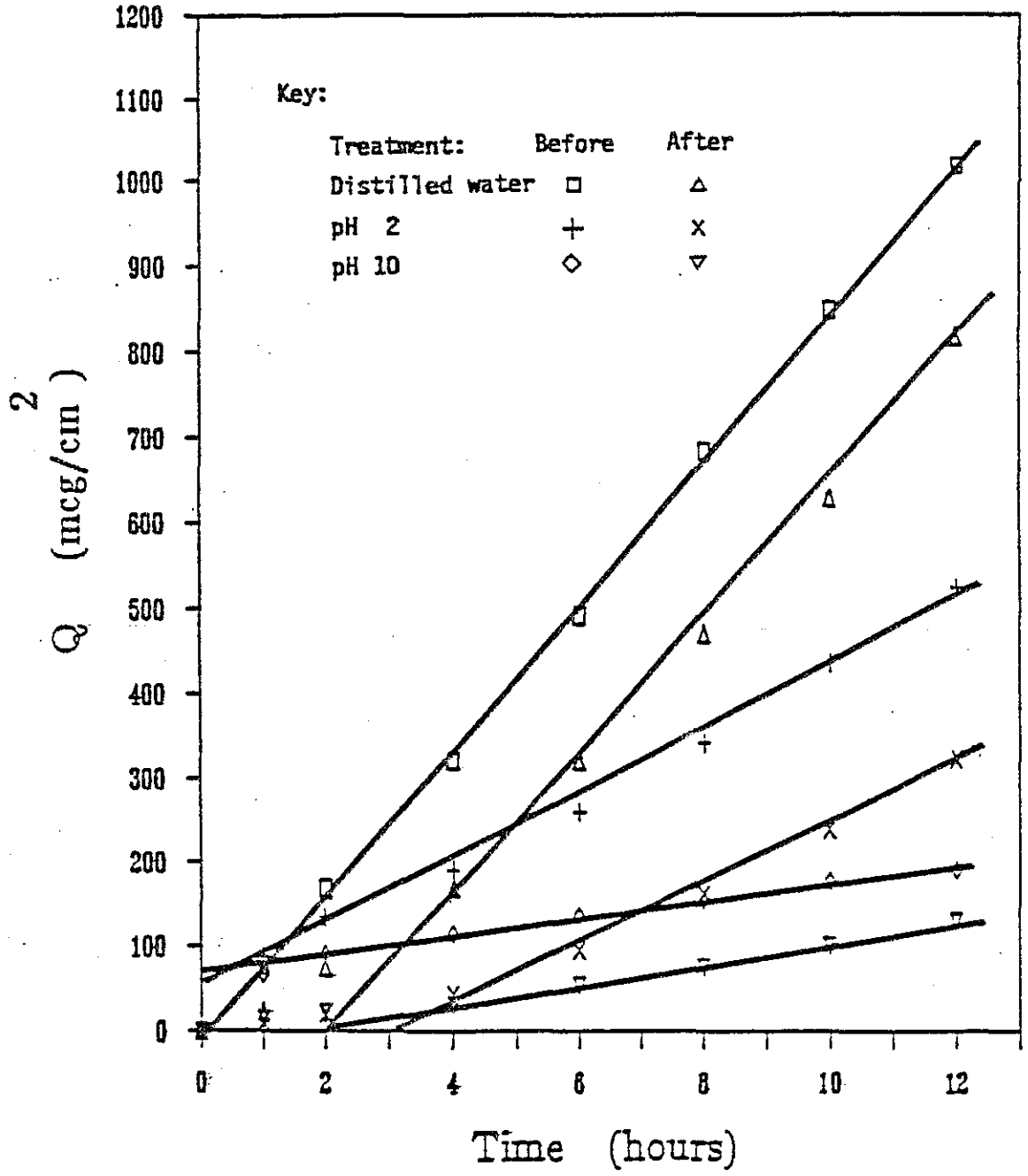

Figure 14. Effect of pH of the vehicle on human cadaver skin.

slight curvature in the pre-steady state region of the penetration curves for all treated samples in Figure 14 suggested that this difference was possibly due to a delay in the attainment of steady-state resulting from partial dehydration of the skin brought about by osmotic effects of the solutions on both sides of the skin. Our interpretation is consistent with the observations of early investigators (113-115) that hydration of the skin plays a significant role in percutaneous penetration of drugs. From this study, it was concluded that exposure of the epidermal side of the skin to isoproterenol HCl suspension in water, pH 2 and pH 10 buffer solutions and simultaneous exposure to the dermal side of the same skin specimen to normal saline for a period of 12 h did not significantly change the flux.

Effect of pH on Percutaneous Penetration of Isoproterenol HCl

Having established the effect of pH and duration of study on the skin, the same experimental design was used to study the effect of Azone on skin permeability towards various ionic and nonionic forms of isoproterenol HCl under varying conditions of pH. Each experimental set (sets 1-5) included two penetration studies (identified

as a and b) on the same sample of skin at each pH before and after treatment with Azone. Figures 15, 17, 19, 21, and 23 and Tables XII, XIV, XVI, XVIII, and XX represent flux data before treatment with Azone while Figures 16, 18, 20, 22, and 24 and tables XIII, XV, XVII, XIX, and XXI represent the flux data after Azone treatment. The experimental design of each set, enabled comparison of fluxes at four pH values to that of the control. Since initial drug concentrations were not identical but only approximately similar within each study, comparison of the fluxes was suggestive but not conclusive in indicating the direction of change in flux with increasing pH. These data suggested maximum flux between pH 8-9.5 in agreement with predictions based upon pH-pKa relationship. The concentration of neutral (uncharged and zwitterionic) species would be maximum around pH of 9. The neutral species were found to have higher permeability coefficients compared to anionic and cationic species. The neutral, cationic and anionic species have permeability coefficients of 0.2098×10^{-3} cm/h, 0.1570×10^{-4} cm/h and -0.8665×10^{-3} cm/h respectively. as calculated from equation 8 and listed in the Table XXII.

$$J = (Kp \cdot C)^{ca} + (Kp \cdot C)^{an} + (Kp \cdot C)^{n} \qquad \text{(Eq. 8)}$$

TABLE XII

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 1a.

	Concentration, mg/ml	Flux (J),[1] mcg/cm^2/h	Correlation Coefficient, r
Control[2]	-	17.95 ± 1.05	0.9973
pH 1.9	266.1	19.59 ± 6.03	0.9949
pH 8.5	250.6	24.71 ± 2.24	0.9988
pH 8.9	225.5	20.47 ± 3.51	0.9957
pH 9.5	302.9	26.47 ± 4.03	0.9966

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

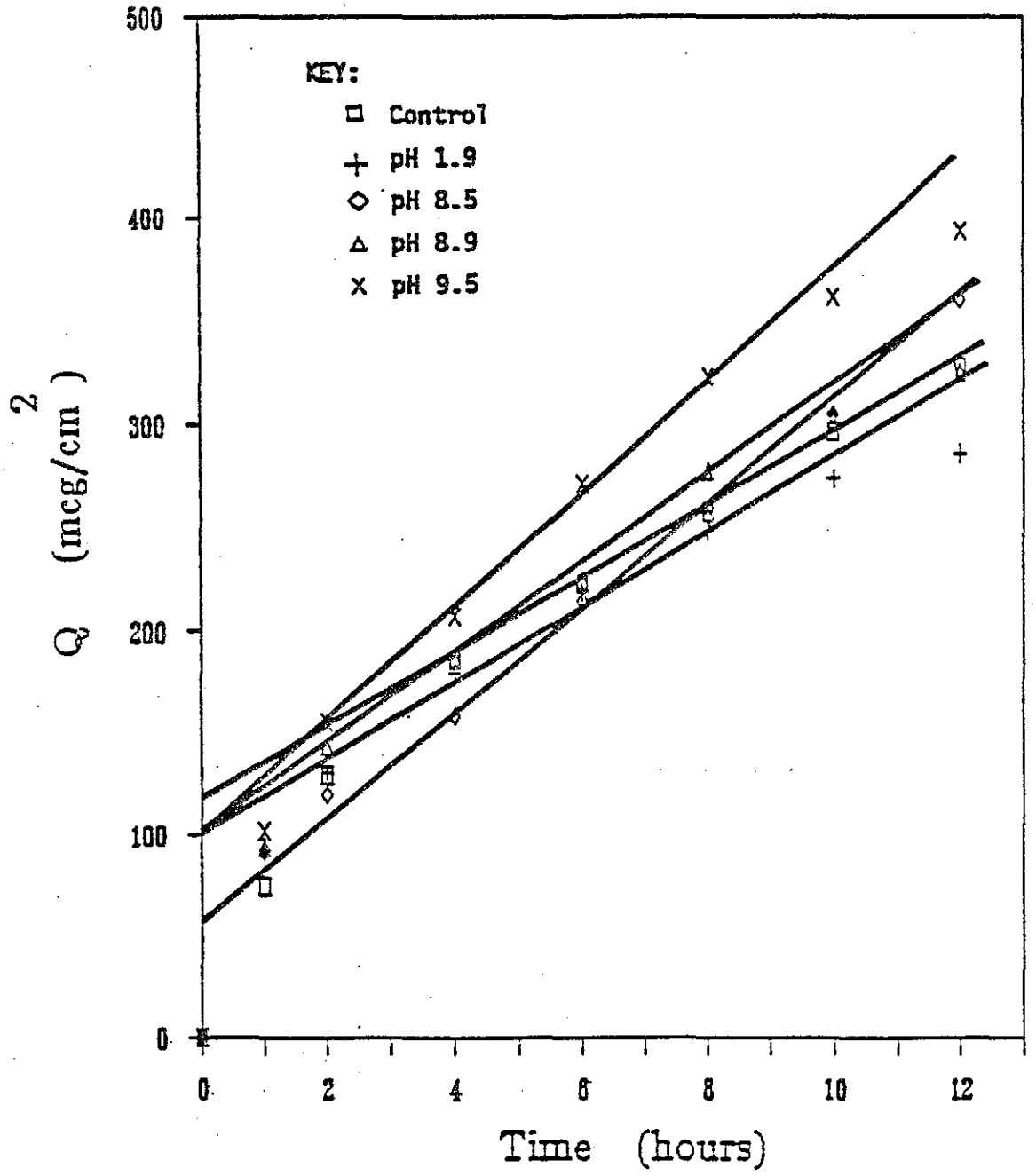

Figure 15. Effect of pH on in vitro percutaneous penetration of isoproterenol HCl. Set 1a.

TABLE XIII

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 1b

	Concentration, mg/ml	Flux (J), [1] mcg/cm^2/h	Correlation Coefficient, r
Control [2]	-	12.04 ± 1.02	0.9989
pH 1.9	270.2	25.75 ± 10.78	0.9907
pH 8.5	284.9	50.74 ± 17.56	0.9936
pH 8.9	248.2	41.46 ± 10.11	0.9968
pH 9.5	85.8	34.85 ± 1.16	0.9994

[1] The tabulated flux values are reported ± 95% confidence limits

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

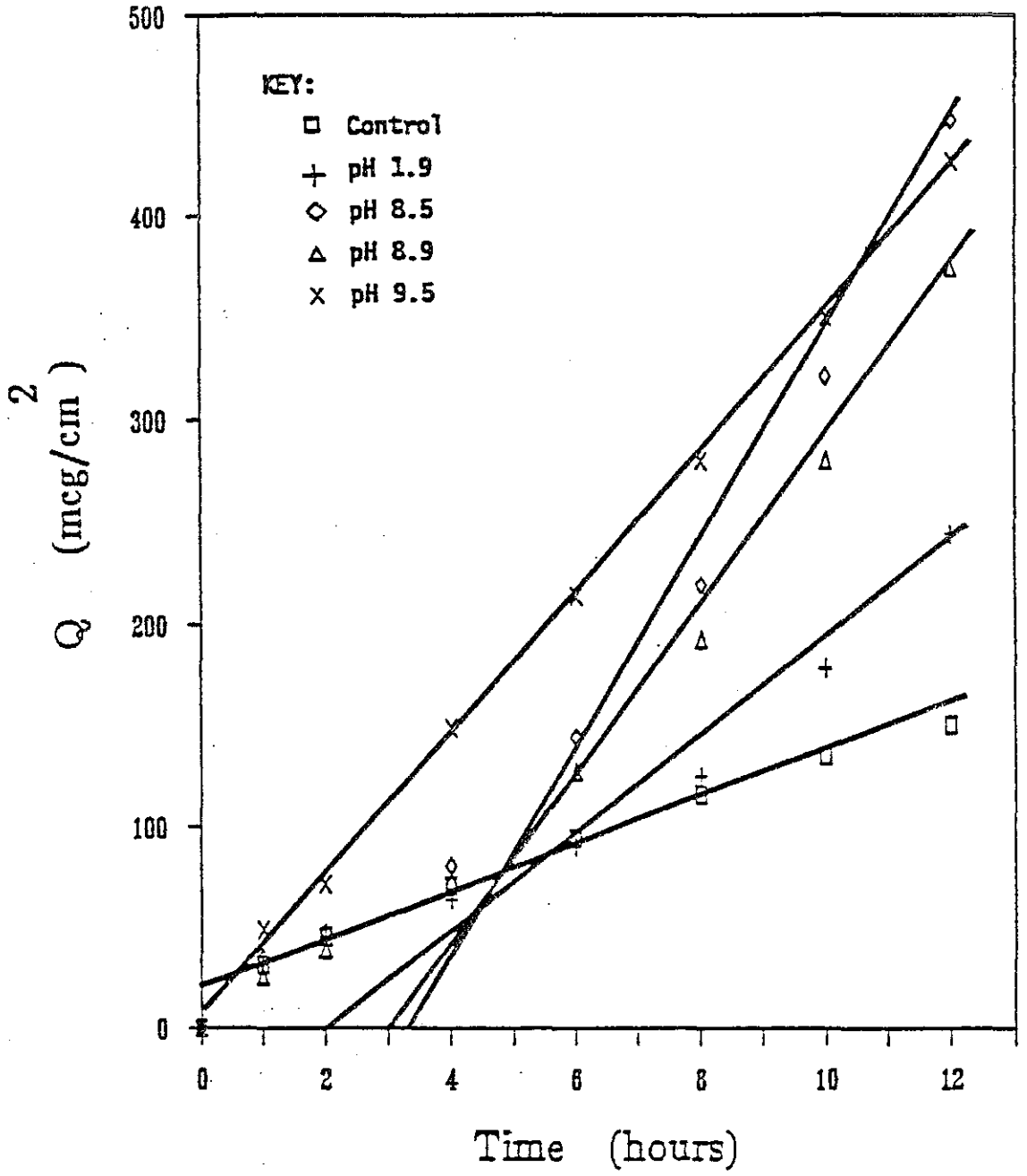

Figure 16. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 1b.

TABLE XIV

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 2a.

	Concentration, mg/ml	Flux (J), [1] $mcg/cm^2/h$	Correlation Coefficient, r
Control [2]	-	12.16 ± 3.35	0.9809
pH 2.0	287.6	12.47 ± 4.10	0.9843
pH 8.2	233.5	53.47 ± 3.41	0.9968
pH 8.5	160.1	21.39 ± 2.75	0.9979
pH 8.8	230.7	52.72 ± 6.21	0.9979

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

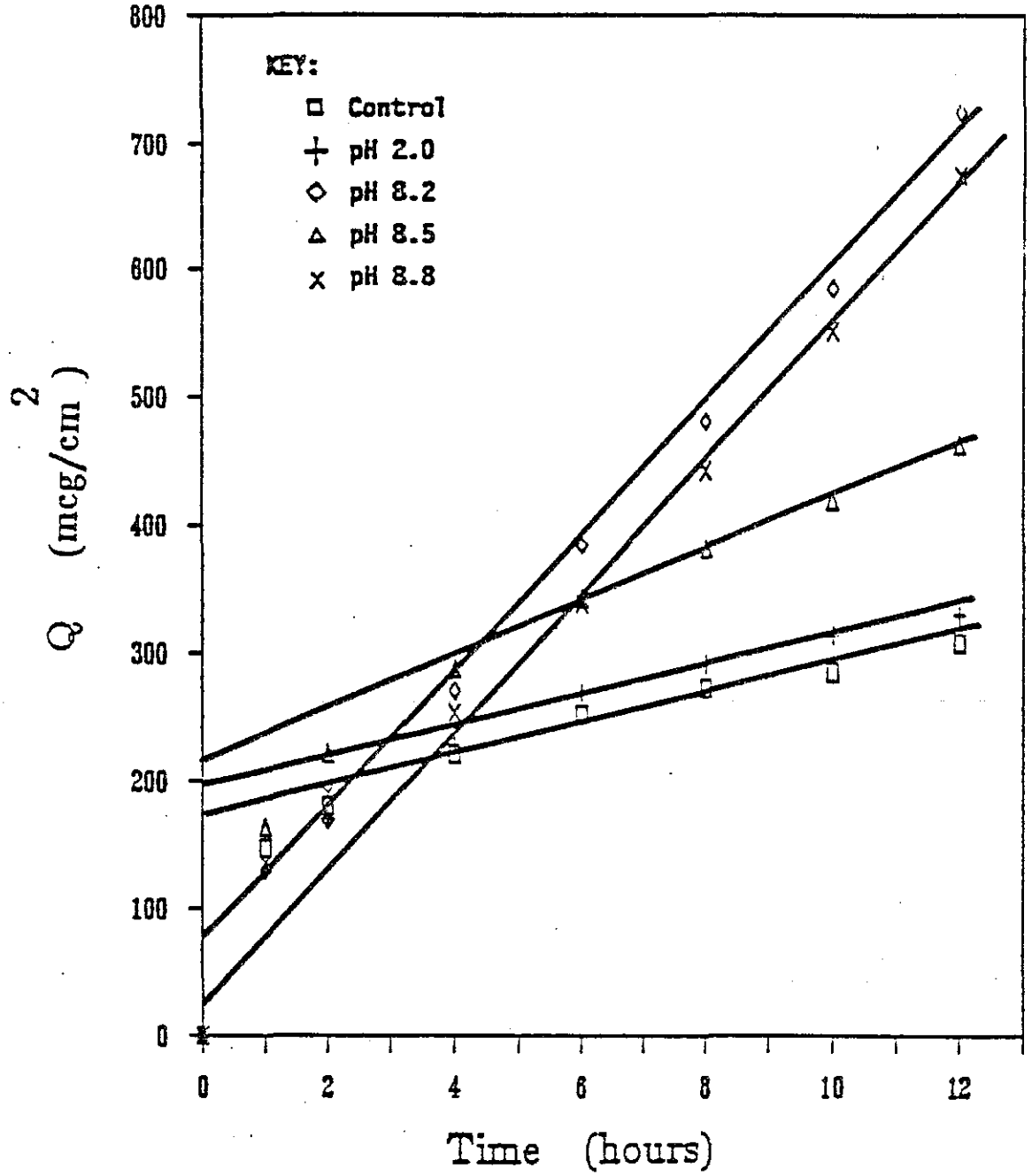

Figure 17. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 2a.

TABLE XV

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 2b.

	Concentration, mg/ml	Flux (J),[1] $mcg/cm^2/h$	Correlation Coefficient, r
Control[2]	-	13.67 ± 0.64	0.9992
pH 2.0	261.2	69.11 ± 41.56	0.9810
pH 8.2	279.5	112.53 ± 19.19	0.9984
pH 8.5	199.5	99.12 ± 7.51	0.9997
pH 8.8	243.1	198.71 ± 5.86	0.9999

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

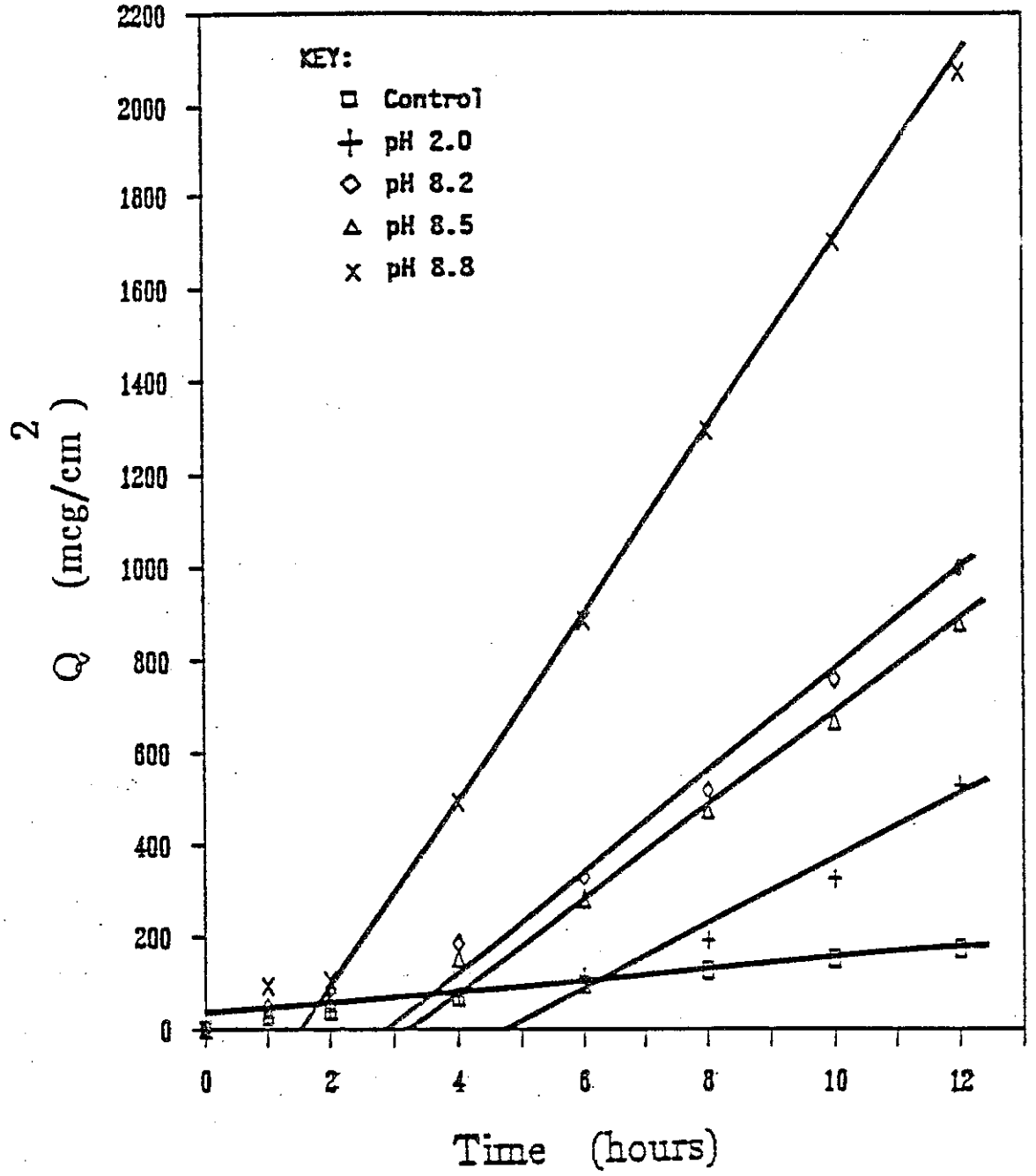

Figure 18. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 2b.

TABLE XVI

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 3a.

	Concentration, mg/ml	Flux (J), mcg/cm^2/h [1]	Correlation Coefficient, r
Control [2]	-	14.05 ± 3.55	0.9907
pH 2.0	83.9	15.58 ± 0.72	0.9997
pH 8.0	80.3	22.30 ± 2.69	0.9979
pH 8.5	80.3	23.26 ± 2.31	0.9995
pH 8.9	78.4	25.66 ± 2.76	0.9983

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

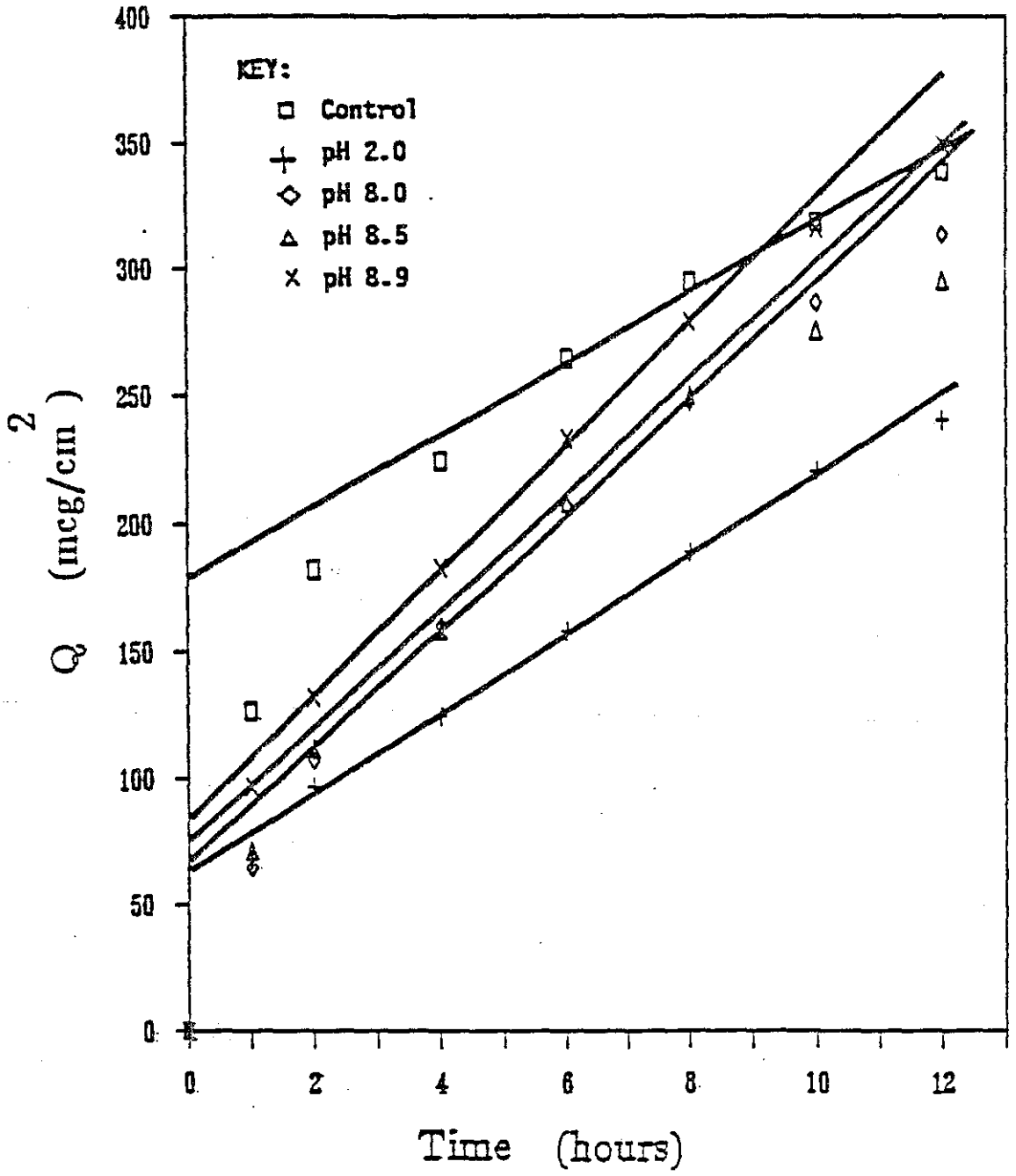

Figure 19. Effect of pH on in vitro percutaneous penetration of isoproterenol HCl. Set 3a.

TABLE XVII

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 3b.

	Concentration, mg/ml	Flux (J), [1] $mcg/cm^2/h$	Correlation Coefficient, r
Control [2]	-	12.79 ± 2.37	0.9950
pH 2.0	95.0	10.35 ± 1.43	0.9951
pH 8.0	97.7	31.16 ± 7.37	0.9970
pH 8.5	77.5	37.06 ± 9.35	0.9966
pH 8.9	74.8	58.15 ± 10.85	0.9981

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

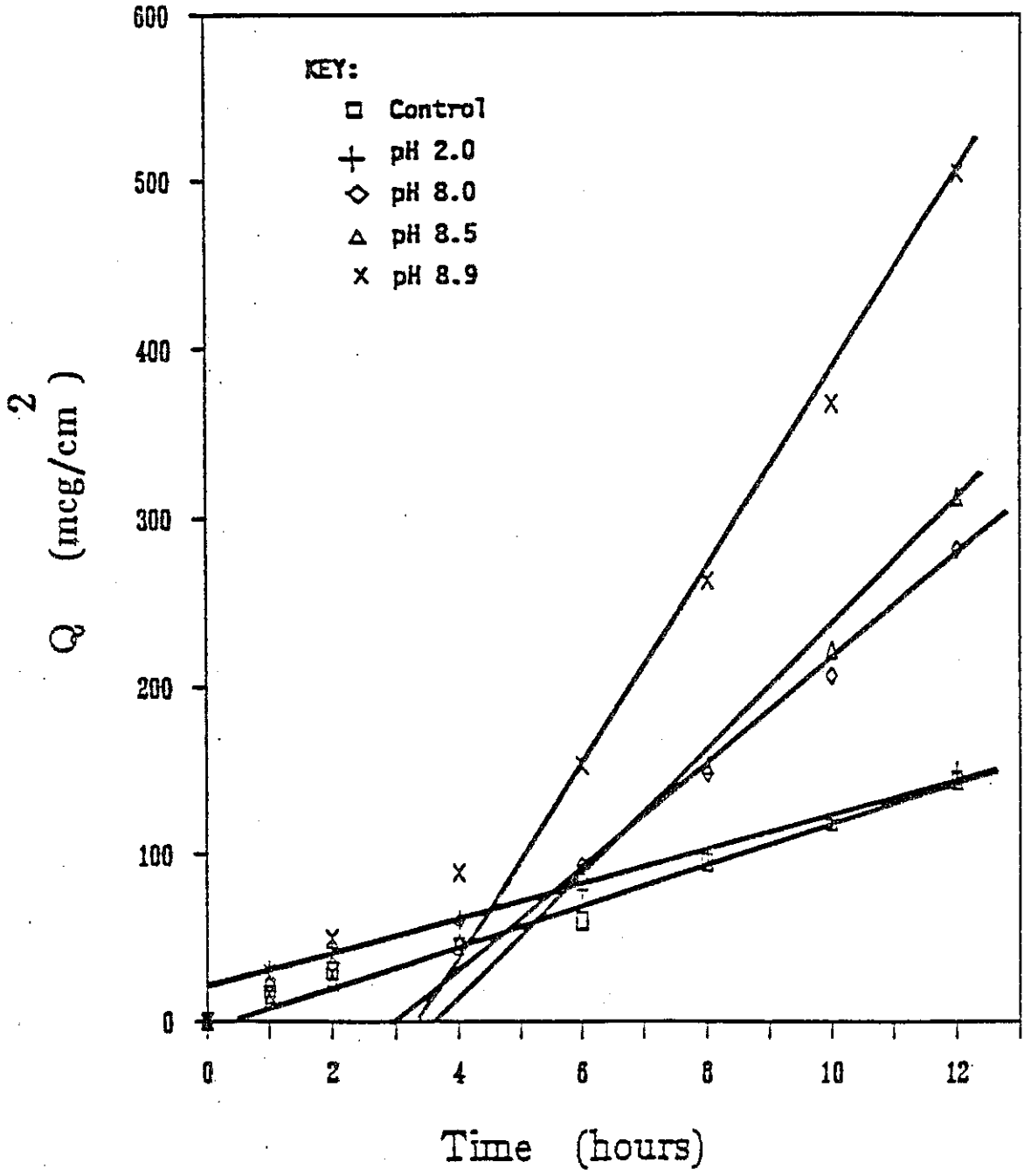

Figure 20. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 3b.

TABLE XVIII

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 4a.

	Concentration, mcg/ml	Flux (J), mcg/cm^2/h[1]	Correlation Coefficient, r
Control[2]	-	24.25 ± 3.34	0.9972
pH 2.0	98.1	25.87 ± 3.90	0.9966
pH 7.9	106.3	19.06 ± 3.57	0.9948
pH 8.5	114.6	23.45 ± 1.42	0.9990
pH 9.1	103.1	23.84 ± 3.01	0.9976

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

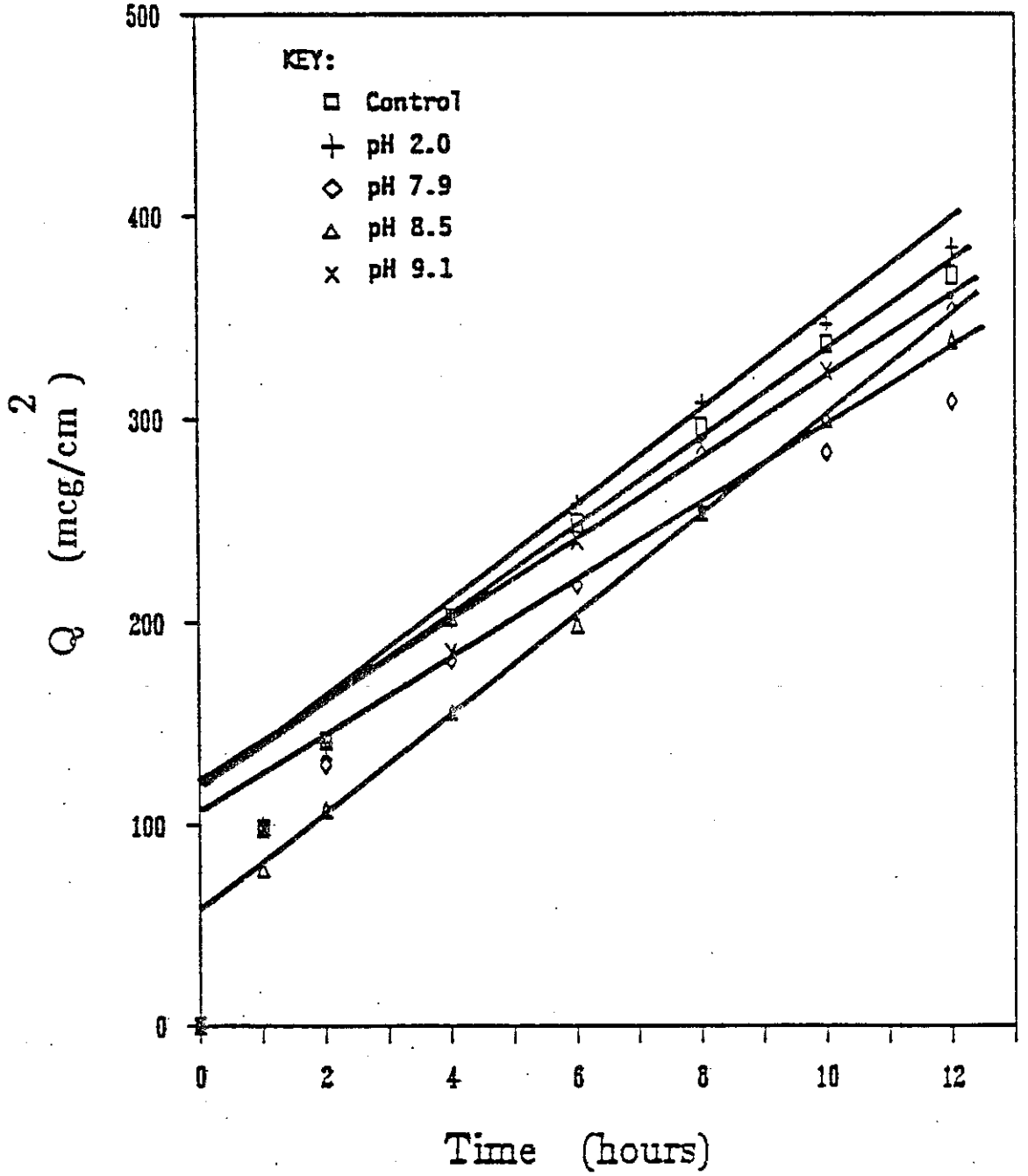

Figure 21. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 4a.

TABLE XIX

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 4b.

	Concentration, mg/ml	Flux (J)[1], mcg/cm^2/h	Correlation Coefficient, r
Control[2]	-	36.84 ± 2.29	0.9994
pH 2.0	100.9	15.90 ± 1.22	0.9985
pH 7.9	106.9	23.69 ± 3.12	0.9974
pH 8.5	110.6	80.91 ± 5.09	0.9998
pH 9.1	106.9	95.54 ± 13.01	0.9990

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

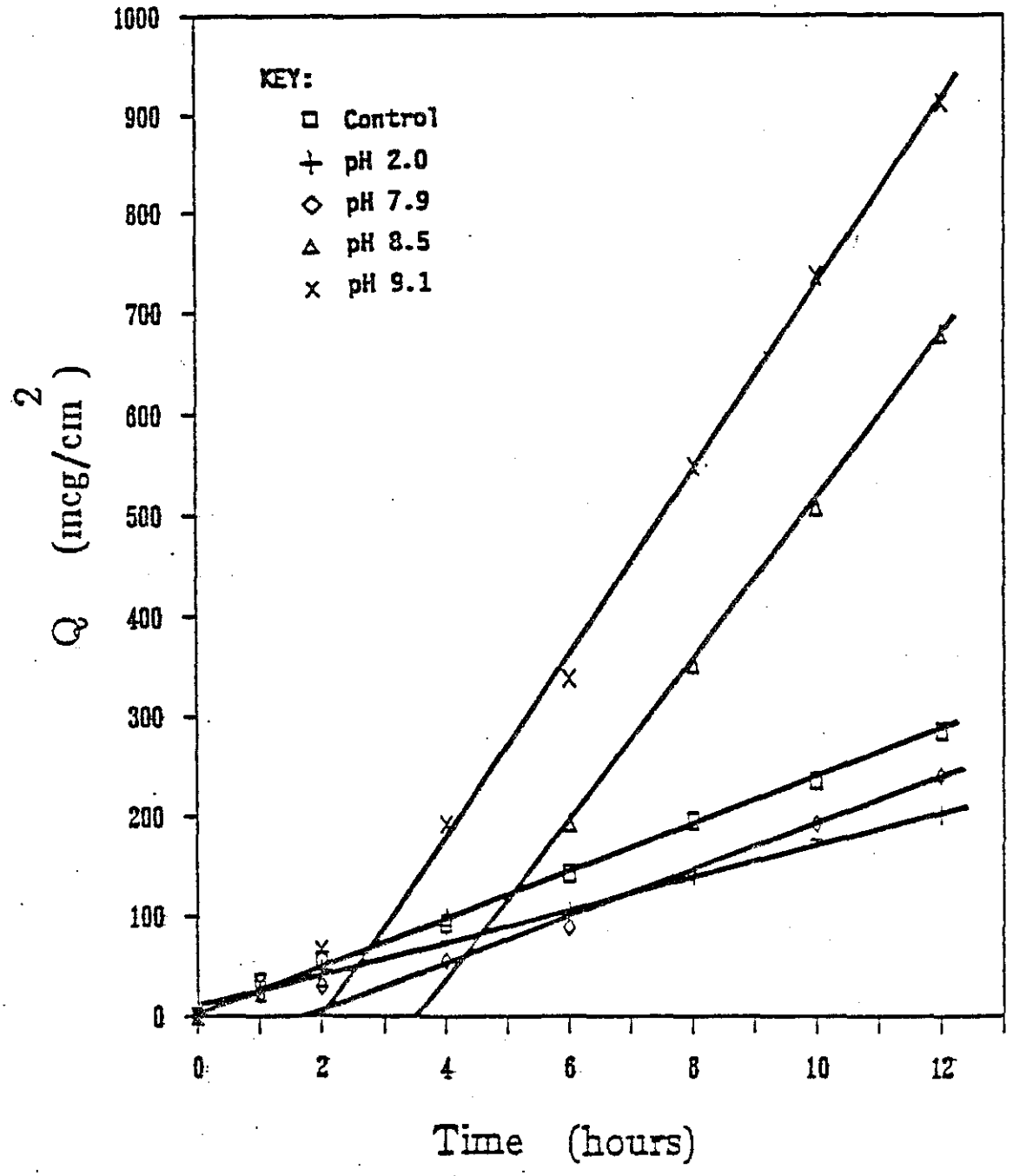

Figure 22. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 4b.

TABLE XX

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 5a.

	Concentration, mg/ml	Flux (J), [1] mcg/cm^2/h	Correlation Coefficient, r
Control [2]	-	16.11 ± 4.20	0.9972
pH 2.0	100.3	11.57 ± 4.37	0.9795
pH 6.9	102.2	10.56 ± 6.35	0.9810
pH 7.9	106.2	23.25 ± 1.39	0.9995
pH 8.5	105.6	26.03 ± 4.67	0.9953

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

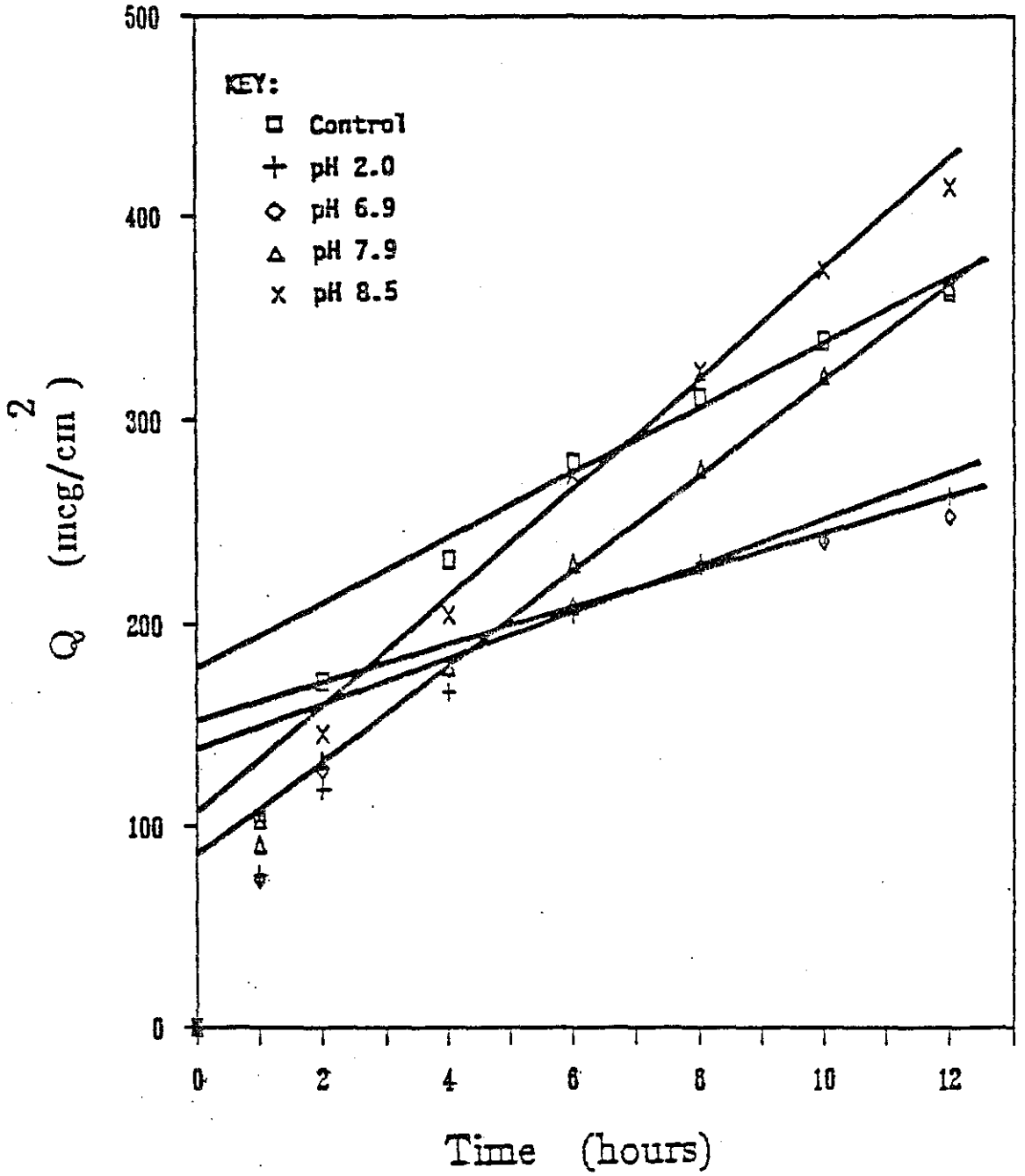

Figure 23. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 5a.

TABLE XXI

Effect of pH on *In Vitro* Percutaneous Penetration of Isoproterenol HCl: Data Set 5b.

	Concentration, mg/ml	Flux (J),[1] $mcg/cm^2/h$	Correlation Coefficient, r
Control[2]	-	15.89 ± 0.97	0.9981
pH 2.0	92.1	34.43 ± 1.39	0.9998
pH 6.9	90.3	12.39 ± 1.90	0.9884
pH 7.9	103.2	19.06 ± 1.23	0.9994
pH 8.5	99.9	23.70 ± 1.12	0.9996

[1] The tabulated flux values are reported ± 95% confidence limits.

[2] Suspension of isoproterenol HCl in distilled water, pH 3.9.

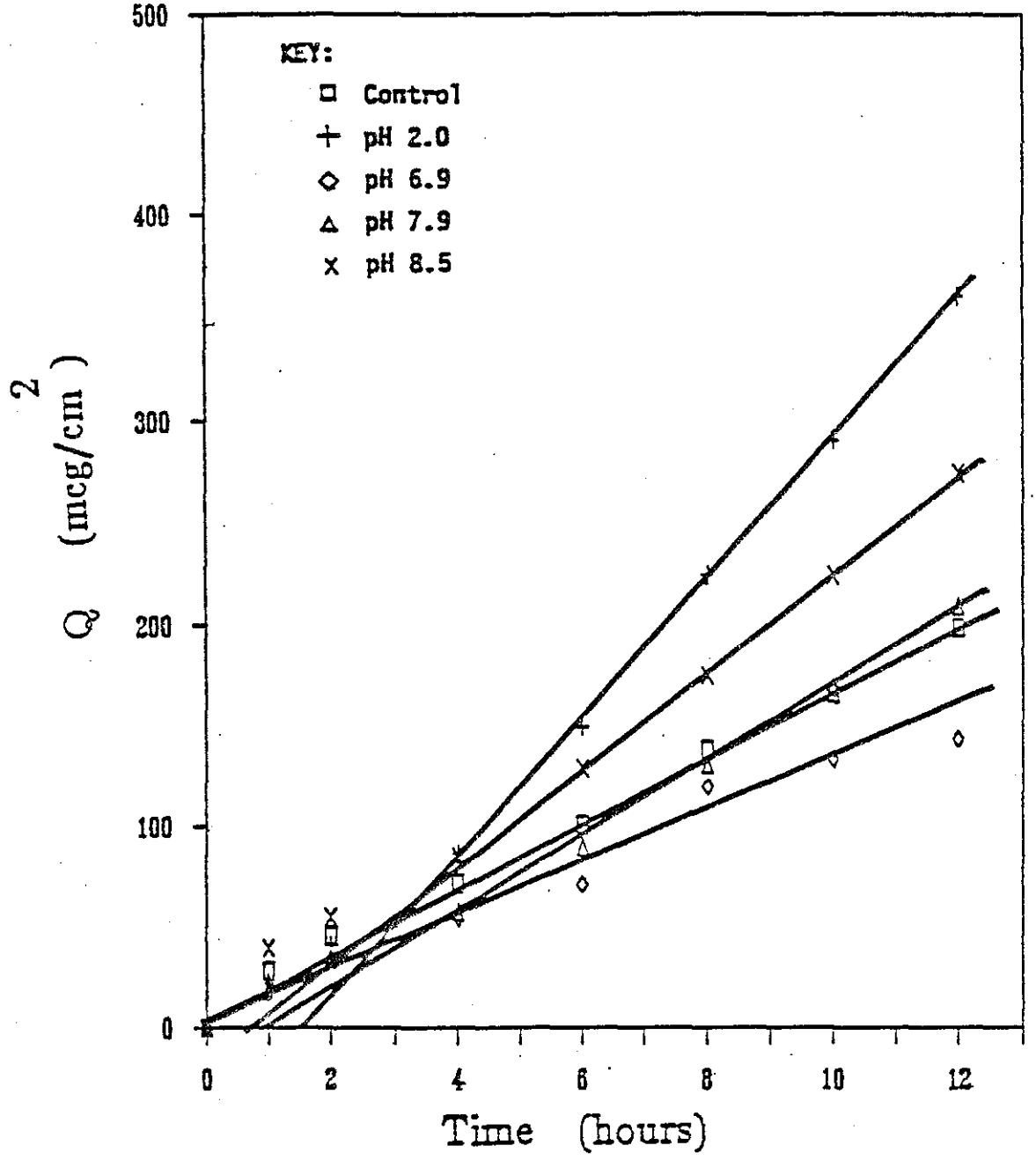

Figure 24. Effect of pH on <u>in vitro</u> percutaneous penetration of isoproterenol HCl. Set 5b.

TABLE XXII

Permeability Coefficients of Various Species of Isoproterenol HCl

	Permeablity Coefficient, cm/h		
	Neutral (K_p)	Cation (K_{pca})	Anion (K_{pan})
Untreated Skin	0.000210 ± 0.000154	0.000016 ± 0.000068	-0.000866 ± 0.000877
Treated Skin[1]	0.000840 ± 0.000724	0.000170 ± 0.000193	0.008091 ± 0.015448

[1] The skin was pretreated with pure Azone for 60 minutes.

When the skin was pretreated with Azone, similar results were seen. There was an increase in the rate of percutaneous penetration with increasing pH (Tables XIII,V,VII,IX,XXI; Figures 16,18,20,22,24). Table XXII also lists the permeability coefficients of different species of isoproterenol HCl through Azone-pretreated human cadaver skin. These are 0.8395×10^{-3} cm/h for the neutral species, 0.1701×10^{-3} cm/h for the cationic species and 0.8091×10^{-2} cm/h for the anionic species, respectively. From a comparison of these values with those obtained from the untreated skin, it can be seen that Azone treatment enhanced the percutaneous penetration of all species of isoproterenol HCl, although the anionic species was favored the most. Previous investigation BenKorah et al. (65) had established that Azone enhances percutaneous penetration of both hydrophilic and hydrophobic molecules by lowering the skin's resistance to diffusion of penetrant molecules. The results of the present investigation not only confirms BenKorah's work but further extends our understanding of the mechanism of penetration enhancement by Azone by revealing that an anionic character is a more desirable attribute of hydrophilic molecules. Although there are no other literature citations substantiating our findings, Hadgraft et al.(67) recently reported

similar observations while studying penetration of salicylates through a filter paper impregnated with isopropyl myristate.

Paired t-Tests on the experimental flux values and the flux values calculated using the permeability coefficients from Table XXII for both, untreated and Azone-pretreated human cadaver skin showed no significant difference ($P > 1.0$) (Table XXIII). This was further confirmed by an excellent correlation between the calculated flux and experimental flux [r (untreated skin) = 1.000, r (Azone-pretreated) = 0.999] as seen in the regression plots (Figures 25-26). Furthermore, these results validated the hypothesis (equation 8) and assumptions that were made therein.

The flux values for untreated and Azone-pretreated skin were calculated from equation 8 using permeability coefficients from Table XXII and percent concentrations of neutral, cationic and anionic forms of isoproterenol HCl obtained from equation 3,4,5 and 6. These flux values were then plotted as a function of pH and the plot was superimposed on the plot of % concentration of various species of isoproterenol HCl against pH (Figure 27). For attaining maximum flux through untreated and Azone-pretreated skin, it was now possible to see that optimal pH would be 9 and 12 respectively, far beyond the

TABLE XXIII

Experimental and Calculated Flux Values for In Vitro Percutaneous Penetration of Isoproterenol HCl

pH	Flux, mg/cm^2/h			
	Untreated Skin		Treated Skin[1]	
	Experimental	Calculated	Experimental	Calculated
1.9	0.0196	0.0204	0.0258	0.0444
8.5	0.0247	0.0343	0.0507	0.0735
9.0	0.0205	0.0377	0.0415	0.1063
9.5	0.0265	0.0244	0.0348	0.0712
2.0	0.0125	0.0208	0.0691	0.0432
8.2	0.0535	0.0282	0.1125	0.0860
8.5	0.0214	0.0278	0.0991	0.0874
8.8	0.0527	0.0373	0.1987	0.1542
2.0	0.0156	0.0176	0.0103	0.0146
8.0	0.0223	0.0195	0.0312	0.0242
8.5	0.0233	0.0220	0.0371	0.0321
8.9	0.0257	0.0237	0.0581	0.0535
2.0	0.0259	0.0178	0.0159	0.0156
7.9	0.0191	0.0201	0.0237	0.0247
8.5	0.0234	0.0245	0.0809	0.0465
9.1	0.0238	0.0258	0.0955	0.1033
2.0	0.0116	0.0178	0.0344	0.0141
6.9	0.0106	0.0181	0.0124	0.0145
7.9	0.0232	0.0201	0.0191	0.0237
8.5	0.0260	0.0239	0.0237	0.0418

Paired t-Test	P
Untreated Skin: J(exp) vs J(calc)	> 1.0
Treated Skin: J(exp) vs J(calc)	> 1.0

[1] Skin pretreated with pure Azone for 60 minutes.

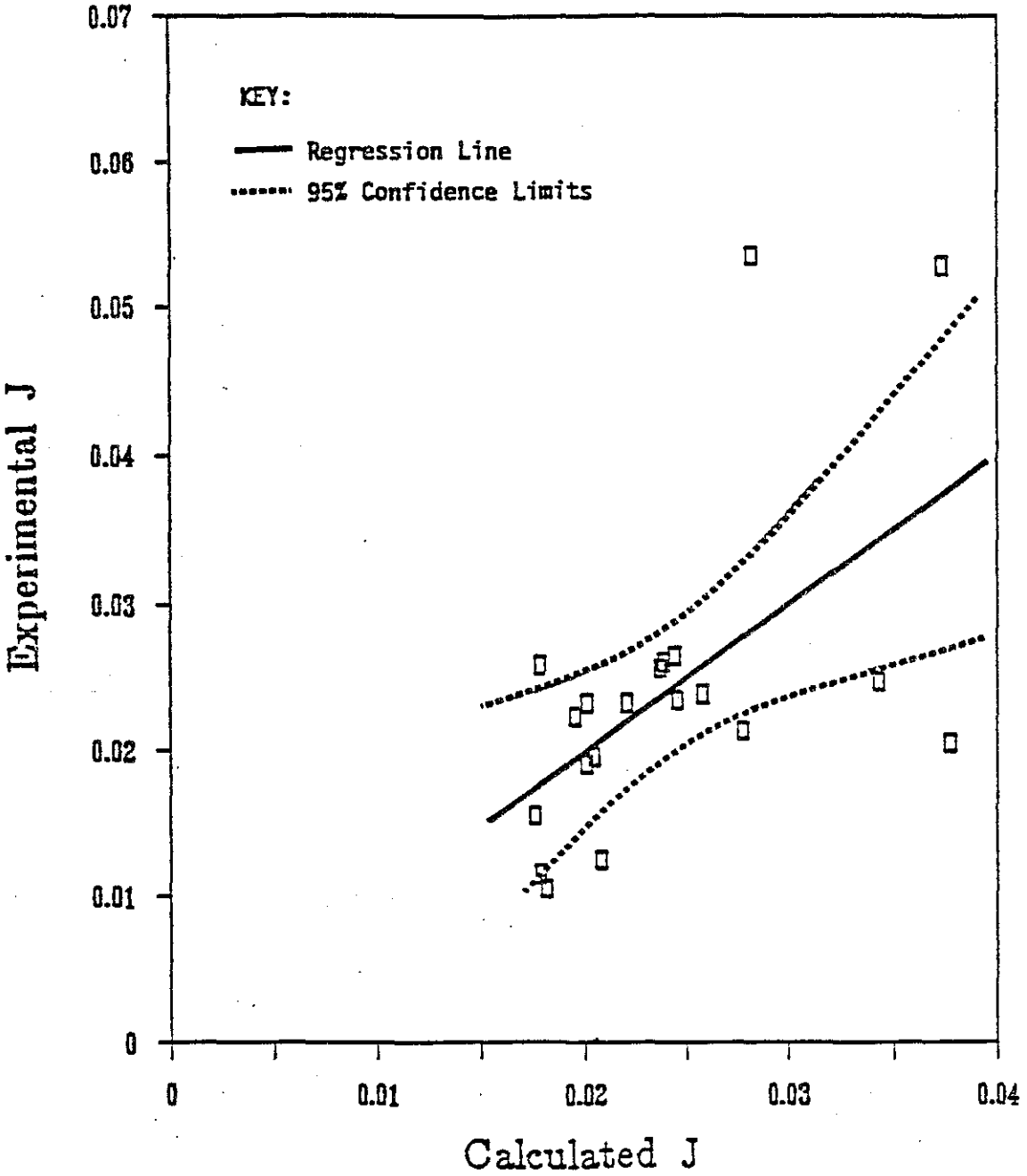

Figure 25. Relationship between calculated and experimental flux through the untreated skin.

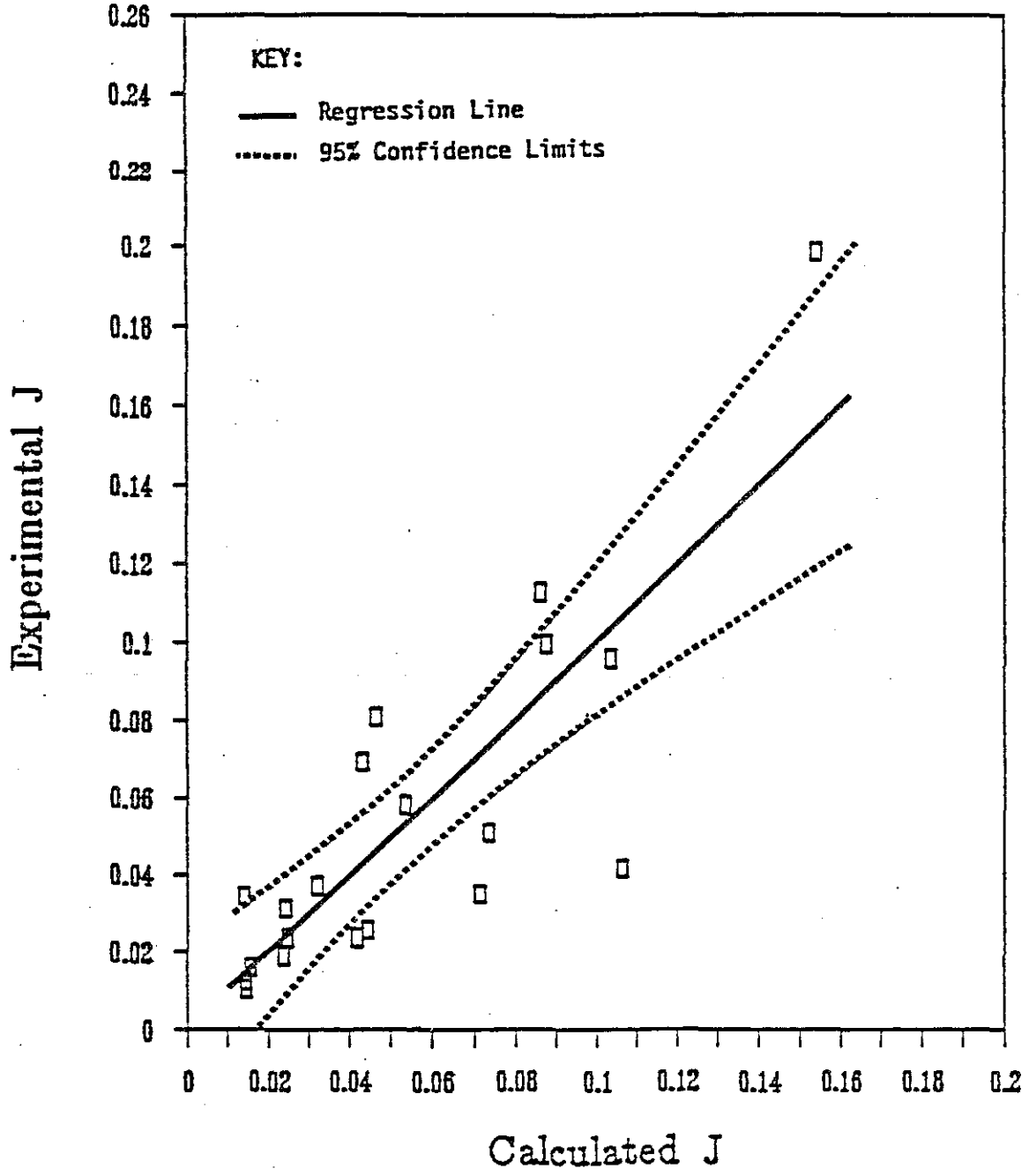

Figure 26. Relationship between calculated and experimental flux through the Azone-pretreated skin.

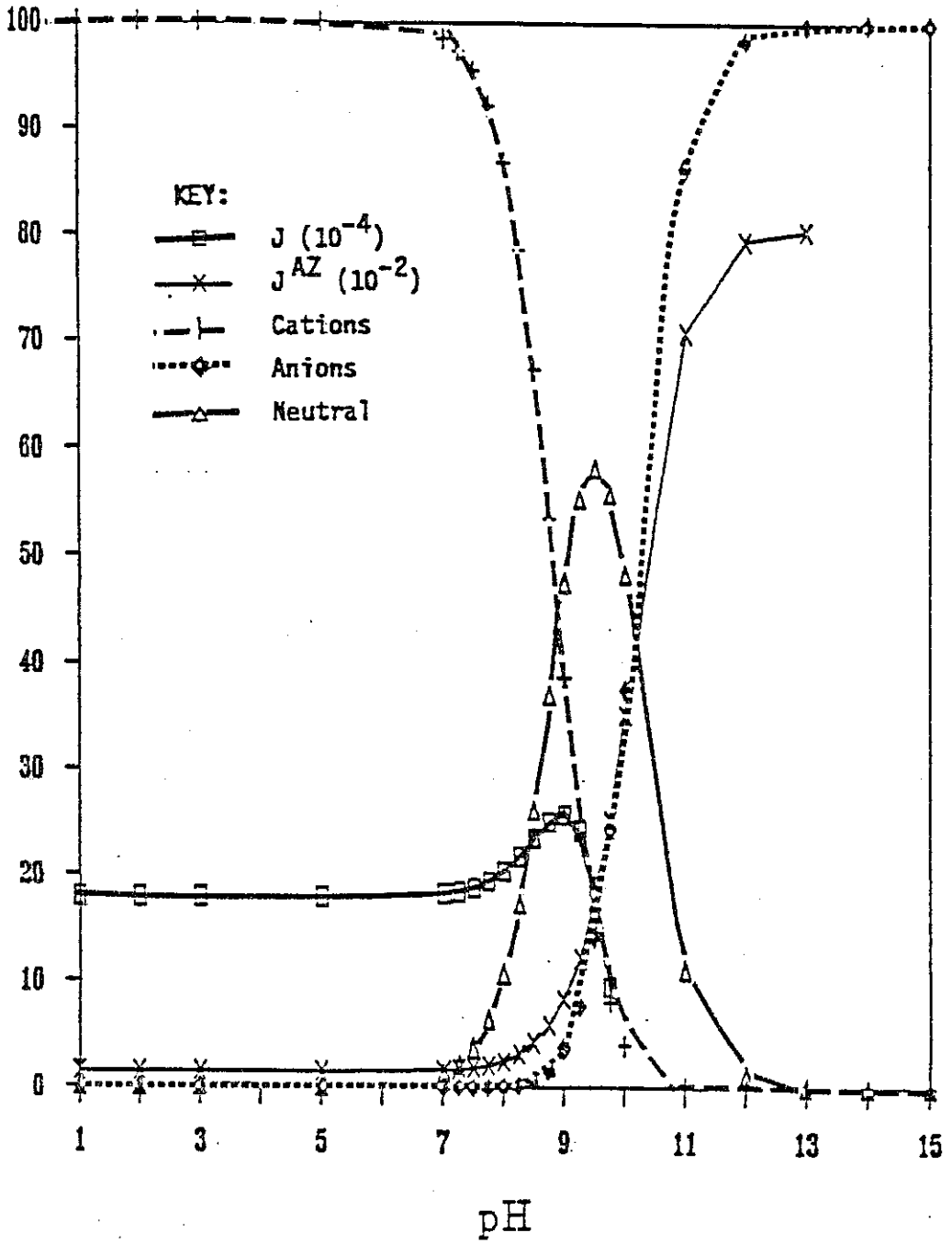

Figure 27. Projected plot of the effect of pH on the total flux through untreated and Azone-pretreated skin and effect of pH on the concentration of various species of isoproterenol HCl.

clinically acceptable range. Nevertheless, this approach can be used for confirming the optimal pH environment for percutaneous penetration and for studying vehicle effects for any ionizable drug molecule.

SUMMARY AND CONCLUSIONS

Much of the theoretical background for percutaneous penetration has been developed through studies of non-ionizing molecules. There are very few literature citations in the area of permeation of ionizable compounds through the skin. Even the penetration enhancing strategies have been tested mainly using non-ionizing compounds. The situation is especially acute with respect to availability of information on the effect of solubility, skin/vehicle partitioning, vehicle composition, pH of the vehicle and penetration enhancers on the permeation of electrolytes.

Depending on the pKa of the compound and pH of the vehicle, a permeating electrolyte exists as an equilibrium mixture of ionized and unionized species in the immediate vicinity of the skin. Although passive diffusion across the skin favors the unionized species of the electrolyte, permeation of ionized forms of the molecules is also possible and cannot be assumed to be negligible, especially when their concentration is high. The permeability coefficients of the unionized molecules have been found to be much greater than those of ionized

species.

In the present work, three important physicochemical properties (stability, solubility and partition coefficient) of an ionizable drug, isoproterenol HCl, have been examined. The stability studies were conducted in distilled water, normal saline, propylene glycol and a series of propylene glycol-water mixtures (10, 20, 40, 60 % v/v) at 22° ± 0.5° and in normal saline in presence and absence of EDTA at 37° ± 0.5° in order to define optimal experimental conditions for subsequent studies. The solubility determination was carried out in distilled water, propylene glycol, various propylene glycol water mixtures (10, 20, 40, 60% v/v) and Azone at 22° ± 0.5°. The partition coefficient determinations were conducted in distilled water, propylene glycol and various propylene glycol-water mixtures (10, 20, 40, 60% v/v) at 22° ± 0.5°. The solubility and partition coefficient results were later utilized to understand and explain the skin penetration of isoproterenol HCl across human cadaver skin.

The effect of propylene glycol concentrations in the vehicle on percutaneous penetration of isoproterenol HCl has been investigated at 0, 20, 60, 100% (v/v) in distilled water. The effect of Azone on skin penetration of isoproterenol HCl has been studied by incorporating 1,

5, 10% (v/v) Azone in 20/80 propylene glycol-water vehicle and also by treating the skin with neat Azone for 3-, 30-, 60- and 720-minute periods prior to application of the drug formulation. Finally, the skin penetration of isoproterenol HCl was investigated under varying conditions of pH through untreated and Azone-pretreated skin. The flux and permeability coefficient values calculated from these studies, were utilized to test the proposed model for penetration of the ionizable drug, isoproterenol HCl, through human cadaver skin under varying conditions of pH in order to understand the penetration enhancing effects of Azone upon the various ionic forms of isoproterenol HCl.

Isoproterenol HCl is very stable (less than 1% decomposition) in water, normal saline, propylene glycol and 10, 20, 40, 60% (v/v) propylene glycol-water mixtures for 24 h at $22^{\circ} \pm 0.5^{\circ}$ in the pH range 1 to 7. The rate of decomposition of isoproterenol HCl in aqueous solutions increased as the pH was increased beyond pH 8.0. The rate of decomposition of isoproterenol HCl in normal saline increased significantly with increase in temperature to $37^{\circ} \pm 0.5^{\circ}$. The solubility of isoproterenol HCl was found to decrease and skin/vehicle partition coefficient of the drug increased with increasing proportions of propylene glycol in the

vehicle, while the product of solubility and partition coefficient appeared to plateau at 20% (v/v) propylene glycol. On the basis of these results and reports of low irritability, 20% (v/v) propylene-glycol in water was predicted as the optimal vehicle for transdermal delivery of isoproterenol HCL.

The results of the penetration study conducted to examine the effect of propylene glycol on percutaneous penetration of isoproterenol HCl appeared to confirm the choice of 20% (v/v) propylene glycol-water system as the optimal solvent for isoproterenol HCl. The increasing permeability coefficients with increases in proportion of propylene glycol in the formulation appear to confirm that propylene glycol facilitates penetration of drug molecules across human skin by lowering the diffusional resistance.

Azone pretreatment was more effective in enhancing percutaneous penetration than incorporating Azone in the vehicle, confirming observations of BenKorah et al.(65). Optimal penetration enhancing effects of Azone were seen, when incorporated at a level of 1% (v/v) in the vehicle and also when the skin was pretreated with pure Azone for 60 minutes prior to application of drug formulation. The flux appeared to reach a maximum around pH 9 in agreement with the predicted optimal pH environment (pH 8.5 to 9.5)

for neutral forms of isoproterenol HCl on the basis of pH-pKa relationship. Permeability coefficients of various species of isoproterenol HCl for penetration through untreated cadaver skin further confirmed this fact. The permeability coefficients were found to be 0.2098×10^{-3} cm/h, 0.1570×10^{-4} cm/h and -0.8665×10^{-3} cm/h for the neutral, cationic and anionic species, respectively. The permeability coefficients of the various species of isoproterenol HCl for skin pretreated with Azone were 0.8395×10^{-3} cm/h, 0.1701×10^{-3} cm/h, 0.8091×10^{-2} cm /h for the neutral, cationic and anionic species, respectively. Comparing these with the permeability coefficients through the untreated skin revealed that Azone, in general, enhanced the penetration of all forms of isoproterenol HCl, although the effect was more pronounced for the anionic species. The latter effect could be explained by formation of an ion-pair between isoproterenol anion and Azone, analogous to the salicylate anion-Azone ion pair proposed by Hadgraft (67).

The paired t-test and regression analyses on the calculated and experimental flux values from untreated and Azone-pretreated human cadaver skin confirmed the validity of the proposed model (equation 8) and its underlying assumptions.

$$J = (Kp \cdot C)^{ca} + (Kp \cdot C)^{an} + (Kp \cdot C)^{n} \quad \text{(Eq. 8)}$$

From the results of this investigation, it was evident that although isoproterenol HCl has a poor penetration rate across the skin, it could be administered transdermally in therapeutically significant amounts by either incorporating 1% (v/v) Azone in the 20/80 propylene glycol-water system or pretreating the skin with Azone before application of the same drug formulation. Isoproterenol HCl is administered at 5 mcg/min by intravenous infusion. If this rate of drug delivery is also recognized as clinically acceptable by the transdermal route, then a suspension of isoproterenol HCl in 20/80 propylene glycol-water system would have to be applied over an area of 23 cm^2. Incorporation of 1% (v/v) Azone in the formulation would require an area of application of 19 cm^2 to achieve the same rate of drug delivery. Pretreatment of the skin prior to application of the drug formulation would further reduce the area of application to 12.5 cm^2. Since the pretreatment of skin is more effective in enhancing the percutaneous penetration of isoproterenol HCl, the conventional transdermal delivery system could be modified by

incorporating Azone in the adhesive layer of this system, thereby bringing Azone in contact with the skin prior to the drug molecules. Since considerable intra- and inter-subject variation exists in the skin and isoproterenol HCl is prone to degradation, in vivo studies must be conducted before any conclusive statements can be made.

This investigation further stresses the importance of basic physicochemical studies and the usefulness of rigorous mathematical and graphic analyses of data in designing transdermal drug delivery systems.

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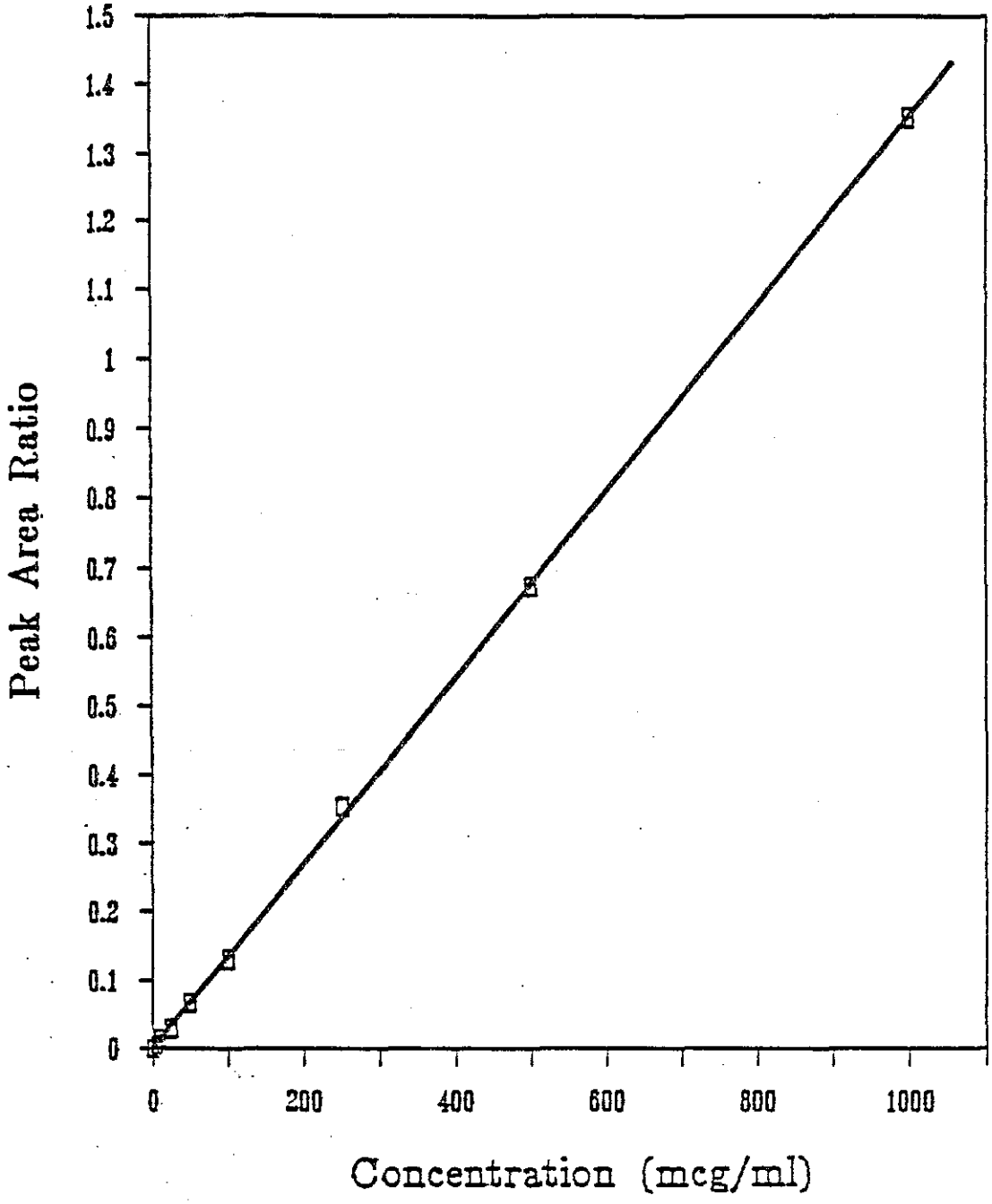
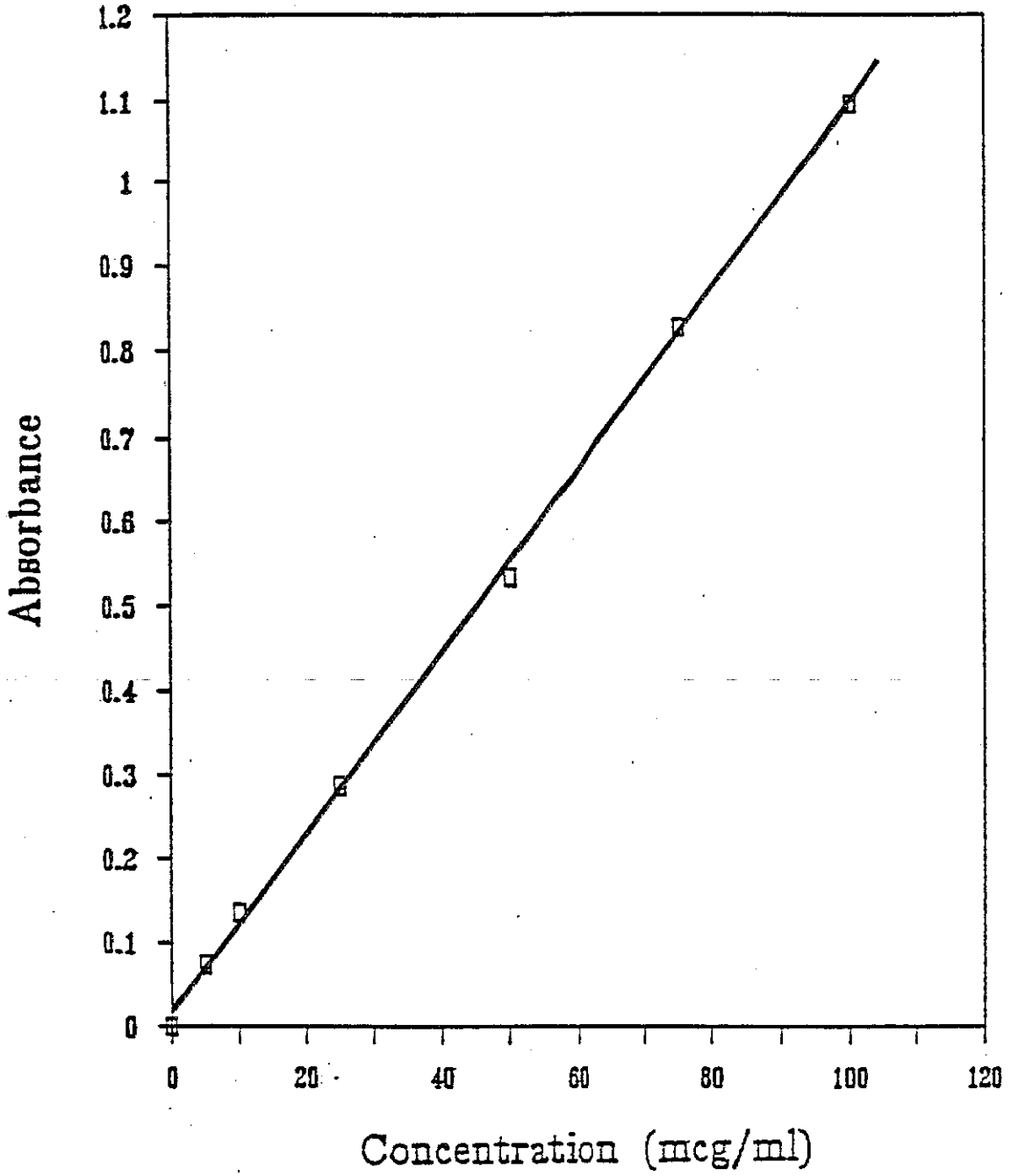


APPENDIX I: HPLC standard plot of peak area ratio against concentration of isoproterenol HCl.
m: 0.0014, b: 0.0, r: 0.9999.



APPENDIX II: Beer´s Law plot of isoproterenol HCl in normal saline.
m: 0.0108, b: 0.0138, r: 0.9996.