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Effect of phase-volume ratio of o/w emulsion vehicles on the activity of a topically applied vasoconstrictor

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Synopsis

This study was intended to test the relationships among phase-volume ratio, transepidermal water loss (TEWL) and skin permeation with the activity of vasoconstrictor emulsions containing hydrocortisone 17-valerate (HCV). Formulas with a phase-volume ratio of 3.59 provide the highest occlusivity (lower TEWL values), while formulas with phase-volume ratio of 0.06 or 0.19 were not occlusive. The major ingredient affecting occlusivity of the o/w emulsion systems is petrolatum, not mineral oil. *In vivo* vasoconstriction activity of a selected HCV 0.2% emulsion relative to several marketed intermediate or moderate corticosteroids was also assessed in 24 normal subjects. The results suggest that the vasoconstriction activity of the newly developed HCV 0.2% emulsion was greater than that of the HCV 0.2% cream, consistent with the *in vitro* skin permeation data. This study also reveals that the permeation rate of o/w emulsions can be increased by increasing occlusivity while still maintaining the cosmetic elegance of o/w emulsions.

INTRODUCTION

If a drug candidate intended for topical medication is to be a useful therapeutic agent, the active moiety must be formulated in a vehicle that is medically rational, physicochemically stable, cosmetically acceptable to the patient, and, above all, able to enhance the drug's biologic activity (1–3). It is known that the clinical efficacy of a topical medication is determined as much by its ability to reach the inflamed tissue as by its inherent activity and the characteristics of the molecule itself. Vehicle design plays a crucial role in the development of an active molecule. In fact, an inappropriate vehicle will diminish a drug's therapeutic potential. Conversely, a suitable base can significantly increase the penetration rate in skin and thereby improve the drug's bioavailability and activity (4,5). In theory, the ideal vehicle should act as an inert medium in which the drug is incorporated in a homogeneous phase.

In this study hydrocortisone 17-valerate (HCV), a nonhalogenated derivative of hydrocortisone shown by Cornell and Stoughton (6) in their clinical evaluation and vasoconstrictor assay to be a mid-potent steroid with superior biological activity over its parent compound, was selected as the candidate molecule. Hydrocortisone 17-valerate 0.2%

cream is a mid-potent steroid used for dermatological disease, e.g., inflammation, psoriasis, and contact dermatitis.

This study was conducted in an attempt to achieve the maximum therapeutic benefit of hydrocortisone 17-valerate by optimizing the vehicle. A standard o/w emulsion system similar to USP XXI Hydrophilic Ointment was adopted as the model formulation. Some modifications were made according to previous kinetic studies (7,8) to ensure that ideal conditions providing maximum thermodynamic activity and chemical stability of hydrocortisone 17-valerate were achieved. Previous studies indicated that 12% propylene glycol in combination with 0.1–0.5% sodium lauryl sulfate at pH 4.7 offers the ideal environment for hydrocortisone 17-valerate 0.2% cream.

Clinically, it is well recognized that an occlusive vehicle enhances the therapeutic efficacy of topical corticosteroids (9–12), possibly by increasing skin hydration which then enhances the penetration of the active drug through the skin. Traditionally, o/w emulsions have been known to be non-occlusive vehicles which provide little or no hydration to the skin as compared to other vehicles, i.e., petrolatum ointments, oils, greases, and w/o emulsions (10–12). Despite the disadvantage of low occlusivity, however, o/w emulsions are still the most popular topical vehicles because of their cosmetic elegance.

The main objective of the study is to explore the possibility of enhancing the clinical efficacy of hydrocortisone 17-valerate by increasing the occlusivity of the o/w emulsion while maintaining its cosmetic elegance.

EXPERIMENTAL

REAGENTS AND MATERIALS

Purified water processed with Milli-Q Water Purification System from Millipore Corp., Bedford, MA, was used throughout the study. USP grade hydrocortisone 17-valerate from Upjohn-Roussel Co., Kalamazoo, MI, was used as active ingredient in formulation design, while the reference standard hydrocortisone 17-valerate for HPLC analysis was obtained from Lark Chemical, Milan, Italy. Internal standard ethyl benzoate was obtained from Aldrich Chemical Co., Milwaukee, WI. HPLC grade acetonitrile purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, was used in the mobile phase during HPLC analysis. ACS grade methanol, octanol, sodium chloride, lithium chloride, magnesium nitrate, and potassium sulfate were obtained from Fisher Scientific Co., Fair Lawn, NJ. Ingredients used in the formulation study were USP grade propylene glycol from Dow Chemical Co., Midland, MI; NF grade sodium lauryl sulfate from Onyx Chemical Co., Jersey City, NJ; NF grade stearyl alcohol from Sherex Chemical Co., Dublin, OH; non-ionic emulsifiers from ICI United States Inc., Wilmington, DE; USP grade dried sodium phosphate from FMC Corp., Philadelphia, PA; NF grade sorbic acid from American Hoechst Corp., New York, NY; and USP grade white petrolatum and mineral oil from Witco Co., Sonneborn Division, New York, NY.

EQUIPMENT

An HP 1090 Liquid Chromatograph with HP 3390 Integrator Recorder from Hewlett Packard, Fairport, NY, was used in the study. A μ-Bondapak C-18 reverse phase column of 30 cm length and 3.9 mm inside diameter with 10 μ particle size from

Waters Associates, Milford, MA, was used. A Metrohn 632 pH meter from Brinkmann Co., Switzerland, was used for pH determination. A Precision penetrometer with 1/10 mm divisions from Precision Scientific Co., Chicago, IL, was used for consistency determination. Evaporimeter EP1 from ServoMed AB, Stockholm, Sweden, equipped with model SR-206 Dual Pen Chart Recorder of Heath Co., Benton Harbor, MI, was used to determine water volatility and transepidermal water loss in the prepared formulas.

FORMULATION DESIGN

Hydrocortisone 17-valerate 0.2%, formulated in a vehicle base similar to USP XXI Hydrophilic Ointment, was chosen as the model formulation. The formula contains 0.2% hydrocortisone 17-valerate as active, 12% propylene glycol as solubilizer, 0.1% sodium lauryl sulfate and 3% non-ionic surfactant as emulsifier, 5% stearyl alcohol as vehicle stabilizer, 0.5% carbopol as thickening agent, and 0.3% sorbic acid as preservative. Other additives were added as needed. Petrolatum, mineral oil, and water were the three variables in the formulation, while the total concentration of the three variables was maintained constant at 78.9%. Each formula was adequately phosphate buffered at its optimal pH 4.70. The variables and the o/w phase-volume ratios (ϕ) of thirty-six formulas are listed in Table I.

The procedure of USP XXI Hydrophilic Ointment was adopted to prepare all the formulations. All prepared formulations were chemically assayed by HPLC method described in USP XXI immediately after preparation.

PHYSICAL STABILITY EVALUATION

Each formula was filled into five 1-oz transparent glass jars and stored at 55°C, 45°C, 35°C, RT (23°C), and −20°C. The samples were examined for physical stability every day for the first seven days, then every week until four months. The judgment used for this evaluation was a self-designed rating scale (see Table II) similar to the method described by Wittern *et al.* (13).

CONSISTENCY DETERMINATION

The consistency of the formula was determined by means of a penetrometer fitted with a polished cone-shaped metal plunger (14). Before measurements were taken, the surface of the formula in each container was made flat with a spatula and then allowed to stand for about ½ hr. Measurements were taken at three different spots, and the resulting values were averaged.

VOLATILITY DETERMINATION

Two methods were used in this study:

Evaporimeter method. This method was based upon water evaporation rate. The evaporimeter was first calibrated by placing the measurement probe in three different humidity standard solutions: lithium chloride (11% relative humidity), magnesium nitrate

Table I
The Variables and the Calculated o/w Phase-Volume Ratios (ϕ) of the Thirty-Six HCV 0.2% Emulsions*

Formula #	% Petrolatum	% Mineral oil	% Water	$\phi = \frac{V_o}{V_w}$
1	0	0	78.9	0.06
2	0	10	68.9	0.19
3	0	20	58.9	0.35
4	0	30	48.9	0.57
5	0	40	38.9	0.88
6	0	50	28.9	1.34
7	0	60	18.9	2.10
8	0	70	8.9	3.59
9	10	0	68.9	0.19
10	10	10	58.9	0.35
11	10	20	48.9	0.57
12	10	30	38.9	0.88
13	10	40	28.9	1.34
14	10	50	18.9	2.10
15	10	60	8.9	3.59
16	20	0	58.9	0.35
17	20	10	48.9	0.57
18	20	20	38.9	0.88
19	20	30	28.9	1.34
20	20	40	18.9	2.10
21	20	50	8.9	3.59
22	30	0	48.9	0.57
23	30	10	38.9	0.88
24	30	20	28.9	1.34
25	30	30	18.9	2.10
26	30	40	8.9	3.59
27	40	0	38.9	0.88
28	40	10	28.9	1.34
29	40	20	18.9	2.10
30	40	30	8.9	3.59
31	50	0	28.9	1.34
32	50	10	18.9	2.10
33	50	20	8.9	3.59
34	60	0	18.9	2.10
35	60	10	8.9	3.59
36	70	0	8.9	3.59

* ϕ values were calculated from V_o/V_w; V_o and V_w are the total volume of oil phase and aqueous phase, assuming the density is equal to 1.

(54.5% relative humidity), and potassium sulfate (97% relative humidity). Approximately 30 mg of the test formula was applied on a microscope slide by means of a microdispenser and was spread to an even layer using a cover glass. The microscope slide was then placed on the probe, and the water evaporation rate (WE) was read to the nearest 0.1 g/m^{2}h after it reached the equilibrium state in approximately three to five minutes.

Weighing method. This method was based upon the percentage of remaining weight after a given period of time. A piece of glass supported by an iron stand was placed on the

Table II
Rating Scale of Physical Stability Evaluation

Rating scale	Physical stability
1	Stable
2	Beginning bleeding
3	Bleeding
4	Beginning separation
5	Separation

surface of a water bath (37 ± 0.1°C). Approximately 50 mg of the test formula was weighed on a known weight of aluminum foil (2 cm × 2 cm) and then placed on the glass. The exact weight of the test material was recorded, and then it was reweighed after ½, 1, 2, 3, and 4 hr. This method was only used for the twelve selected formulas in the *in vitro* skin permeation study and *in vivo* transepidermal water loss determination.

TRANSEPIDERMAL WATER LOSS DETERMINATION (TEWL)

The TEWL of the wrist and forearm of human volunteers was determined by means of the evaporimeter. The TEWL of the bare skin was first recorded before the test formula was applied to the spot. The TEWL was read again right after the test material was spread in an even layer using a small spatula, then continuously read every 15 minutes for 1 ½ hr. During measurement, the skin was left open and undisturbed at ambient conditions (23 ± 2°C, 40 ± 5% RH). TEWL determinations were made only for the selected twelve formulas.

COSMETIC ACCEPTANCE EVALUATION

The twelve selected formulas were evaluated for their cosmetic acceptance using a self-designed rating system (Table III). Five males and five females were asked to score the formulas. Color and appearance were judged by looking directly at the formulas in the jar. By rubbing approximately 0.5 gm of the test formula onto the back of the hand, odor, homogeneity, texture, spreadability, and greasiness were evaluated. Homogeneity was also examined by spreading the formula on a big spatula using another smaller spatula. Finally, washability was scored by washing the applied formula on the hand using warm water (30 ± 3°C). Two-way analysis of variance (ANOVA) was used to analyze the scores obtained for individual and overall characteristics. Whenever the ANOVA results showed that the means were significantly different from each other, the Tukey's Honestly Significant Difference (HSD) test was conducted to obtain more detailed information about the differences among the means. This statistical analysis was done using an IBM 4033 computer.

PREPARATION OF EXCISED HUMAN SKIN

Human skin obtained from autopsy was cleaned of hair and fat with a regular razor blade, scissors, and forceps. The skin was then sectioned by a model 880 freezing microtome (AQ Scientific Instruments, Division of Warner Lambert Tech., Inc., Buf-

Table III
Rating Scale of Cosmetic Acceptance Evaluation

Characteristics \ Rating scale	1	2	3	4	5
Color	Pure white	Off-white	Yellowish	Yellow	Brownish
Appearance	Very shiny	Shiny	Slightly shiny	Dull	Unctuous
Odor	Odorless	Faint odor	Faintly unpleasant	Unpleasant	Extremely unpleasant
Homogeneity	Very smooth	Smooth	Very slightly non-uniform	Slightly non-uniform	Non-uniform
Texture	Very soft	Soft	Slightly stiff	Stiff	Vey stiff/ hard
Spreadability	Very easy to spread (no drag)	Easy to spread	Slightly difficult to spread	Difficult to spread	Very difficult to spread
Greasiness	Non-greasy	Very slightly greasy	Slightly greasy	Greasy	Very greasy
Washability	Very washable	Slightly washable	Very slightly washable	Practically non-washable	Non-washable

falo, NY) to a thickness of approximately 200 μm. The sectioned skin was stored in normal saline solution in a refrigerator until ready to use within 24 hr.

IN VITRO HUMAN SKIN PERMEATION STUDIES

Flat-type 9-mm inside diameter Franz diffusion cells with open top caps were used. Normal saline solution maintained at 37 ± 0.1°C was used as receptor fluid. A piece of section skin was carefully mounted on top of the cell. A known weight of test formula (approx. 50 mg) was applied on the skin surface at the center of the cell covering the entire cell, then the cap was secured in place with a clamp. One hundred and fifty μL of sample was drawn at the following intervals: 1, 4, 8, 24, 26, 28, 30, 32, 48, 50, 52, 54, 56, and 72 hr. The withdrawn volume was replaced with fresh normal saline to maintain the initial volume. The sample solutions were immediately ready for HPLC analysis. The skin specimen was examined visually to ensure that no leakage occured during the experiment.

CALCULATION OF PERMEABILITY CONSTANT

When the cream is present on the surface of the specimen, the concentration of drug in the outer layers of the stratum corneum at equilibrium is higher than the concentration in the receptor fluid. The concentration in the lower layers of the skin remains near zero, since these layers are in contact with a fluid that is being continuously replaced or through which diffusion is relatively rapid. The flux, therefore, is more accurately related to the difference in concentration between the top and the bottom layers of the skin (15). The concentration in the top layers of the skin is determined by the relative solubility of the drug in the vehicle and the skin, i.e., the partition coefficient of drug in the skin (K_m). Fick's law is then expanded to:

$$\frac{dQ}{dt} = \frac{A K_m D C_o}{h} \quad (1)$$

where dQ/dt is the flux; h is the thickness of the skin; Q, the amount of drug diffused into the receptor fluid at time t; D, the diffusion coefficient of the drug in the skin; and A, the area of the drug applied on the skin.

Equation (1) can be rearranged to,

$$K_p = \frac{d\,Q/d\,t}{C_o A} = \frac{K_m D}{h} \quad (2)$$

where K_p is the mean permeability constant.

IN VIVO VASOCONSTRICTOR STUDIES

The vasoconstructor activity of hydrocortisone 17-valerate 0.2% in the finally selected emulsion was evaluated clinically and compared to that of other marketed corticosteroid products in healthy human volunteers. The method used was similar to the vasoconstriction assay described by Burdick *et al.* (16), which is a modification of the bioassay described by McKenzie and Stoughton (17).

Approximately 3 mg of each preparation was applied to 12–16 sites (each 7 mm × 7 mm in size) on the volar surface of the forearms of 24 normal healthy subjects. The materials remained on the skin for six hours, unoccluded but protected from mechanical abrasion by an elevated plastic arm guard. A restricted randomization process, balanced for sites and arms, was used in assignment of preparations to the sites in groups of 8 or 12 subjects. One or two pairs of observers independently evaluated the blanching responses, on a scale of 0 to 3, at 7, 9, 11, 13, and 24 hr after application of the materials, in three separate assays. The results of the vasoconstrictor studies were assayed by using the area under the time-response curves according to the trapezoidal rule. The data were evaluated by analysis of variance, using Duncan's procedure for multiple comparisons with $p \leq 0.05$ for adjacent pairs of means.

RESULTS AND DISCUSSION

FORMULATION EVALUATION

Thirty-six hydrocortisone 17-valerate 0.2% o/w emulsions (see Table I) were studied. The three major ingredients—petrolatum, mineral oil, and water—which most likely affect the occlusivity of the emulsion system were treated as variables in the formulation study while other ingredients were held constant. Preparation of formulations with low water content (8.9% and 18.9%) was found to be difficult, presumably because the amount of emulsifiers in these formulations was insufficient for the high oil content formulas. The combination of three variables gives different phase-volume ratios for formulations (see Table I).

PHYSICAL STABILITY EVALUATION

Based upon the scoring system (see Table II), it was observed that formulas with low water content ($\phi = 3.59$) were not physically stable for three weeks at 55°C, 1 month

at 45°C, and two months at 35°C. Although formula #8 seemed to have better physical stability after two months at different temperatures, it still started to bleed within three months. Formulas containing 18.9% water were physically stable for at least four months. The physical instability of these formulas was likely due to incomplete emulsification observed during the preparation of these formulas. Formulas containing 28.9% or more water were found to be physically stable for at least four months at all storage temperatures.

CONSISTENCY DETERMINATION

Most of the emulsions prepared in the study were highly viscous, and their viscosity and yield values were difficult to measure with a conventional viscometer. A penetrometer was used to measure the consistency of the emulsions. The consistency results are plotted in Figure 1. As can be seen, at constant concentration of petrolatum (or mineral oil), the formulas become high-consistency emulsions as the concentration of mineral oil (or petrolatum) is increased. However, regardless of the water concentration, as soon as the total concentration of the oil phase reaches 60% or more, slight decreases in consistency (slight increases in penetration depth) were observed. This phenomenon might be due to insufficient emulsification power, where the unemulsified oil phase acts as a lubricant causing deeper penetration of the plunger.

VOLATILITY DETERMINATION

Figure 2 represents the data obtained from water evaporation rates determined by an evaporimeter and plotted against the concentration of mineral oil in the formulas. The phase-volume ratio indicated in parentheses also follows the trend in which the water evaporation rate decreases as the phase-volume ratio increases. At a constant concentration of mineral oil, the water evaporation rate decreases inversely with the concentration of petrolatum but is directly proportional to the concentration of water in the formulas.

The trend presented in Figure 2 seemed to agree reasonably well with the results of consistency in Figure 1; however, the water evaporation rate decreased continuously even at low water content level (8.9% and 18.9%), whereas the consistency of the same formulas increased slightly.

Due to the limited source of excised human skin, a small group of formulas was selected for further evaluation. The selection was based on physicochemical stability results and water evaporation rates which suggest that at least 28.9% water might be needed to achieve complete emulsification and at least 40% oil phase is needed to reduce the water evaporation rate. A "one-factor-at-a-time" technique was adopted for formulation optimization. The approach used was to select a center formula (#28) which meets the above two criteria. Two straight lines were drawn perpendicularly across formula #28 as shown in Figure 2 (dotted line). Formulas (#2, #10, #17, #23, and #28—designated as group 1) selected from the vertical line, which represents formulations with different phase-volume ratios from 0.19 to 1.34, were studied first to determine the effect of the o/w phase-volume ratio. Secondly, at a fixed ϕ (1.34) value, formulas (#6, #13, #19, #24, #31, and #28—designated as group 2) from the horizontal line representing formulations with different petrolatum content, were studied for any activity increase that could be further enhanced by increasing the concentration of petro-

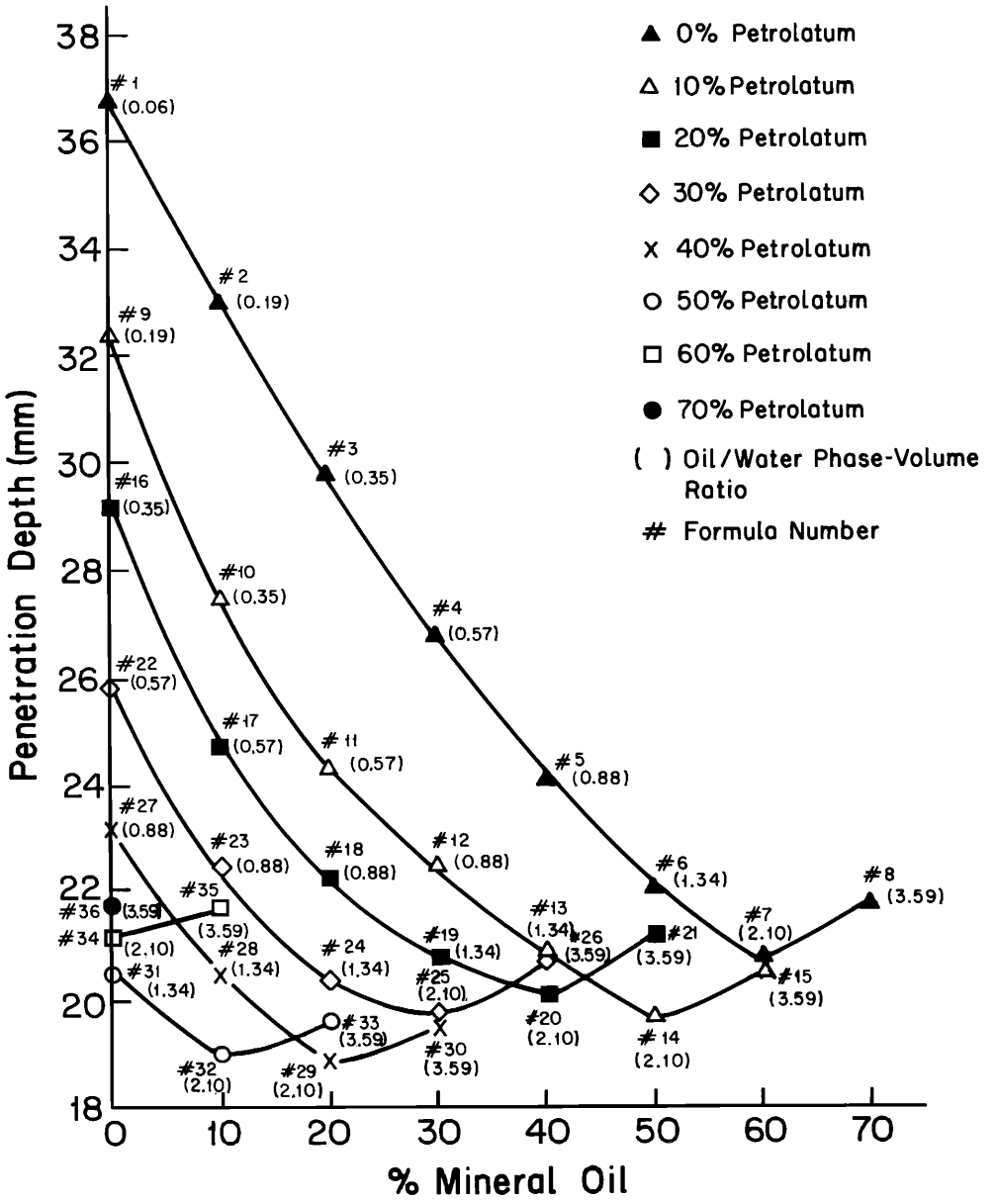

Figure 1. Consistency of the 36 hydrocortisone 17-valerate 0.2% o/w emulsions.

latum or mineral oil. Formulas #32 and #35 were excluded from further studies because of their physical instability. Two formulas representing extreme conditions, #1 and #36, were added to the study for comparison.

A direct weight loss determination was then conducted on the selected twelve formulas in order to quantify the amount due to evaporation loss at ambient conditions. Figure 3

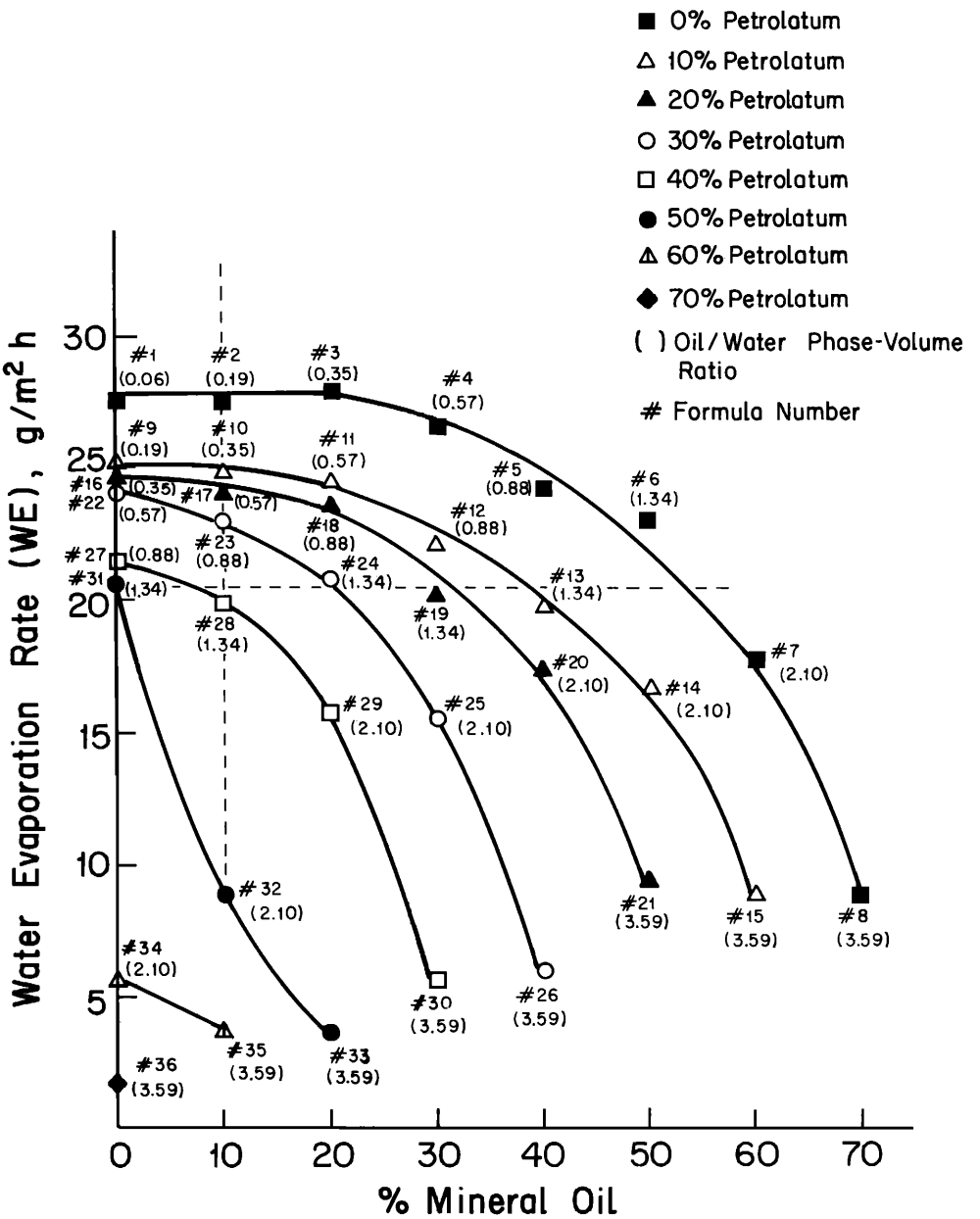

Figure 2. Water evaporation rate (WE) and the percentage of mineral oil in the test o/w emulsions.

demonstrates the volatility of the selected twelve emulsions in four hours at ambient conditions. The results in Figure 3 agree well with those of Figure 2. The formulas with the greatest amount of water have the greatest weight loss.

From a practical viewpoint, the evaporation of water from the skin or emulsions into the atmosphere is a continuous process. The vehicle effect due to water evaporation will

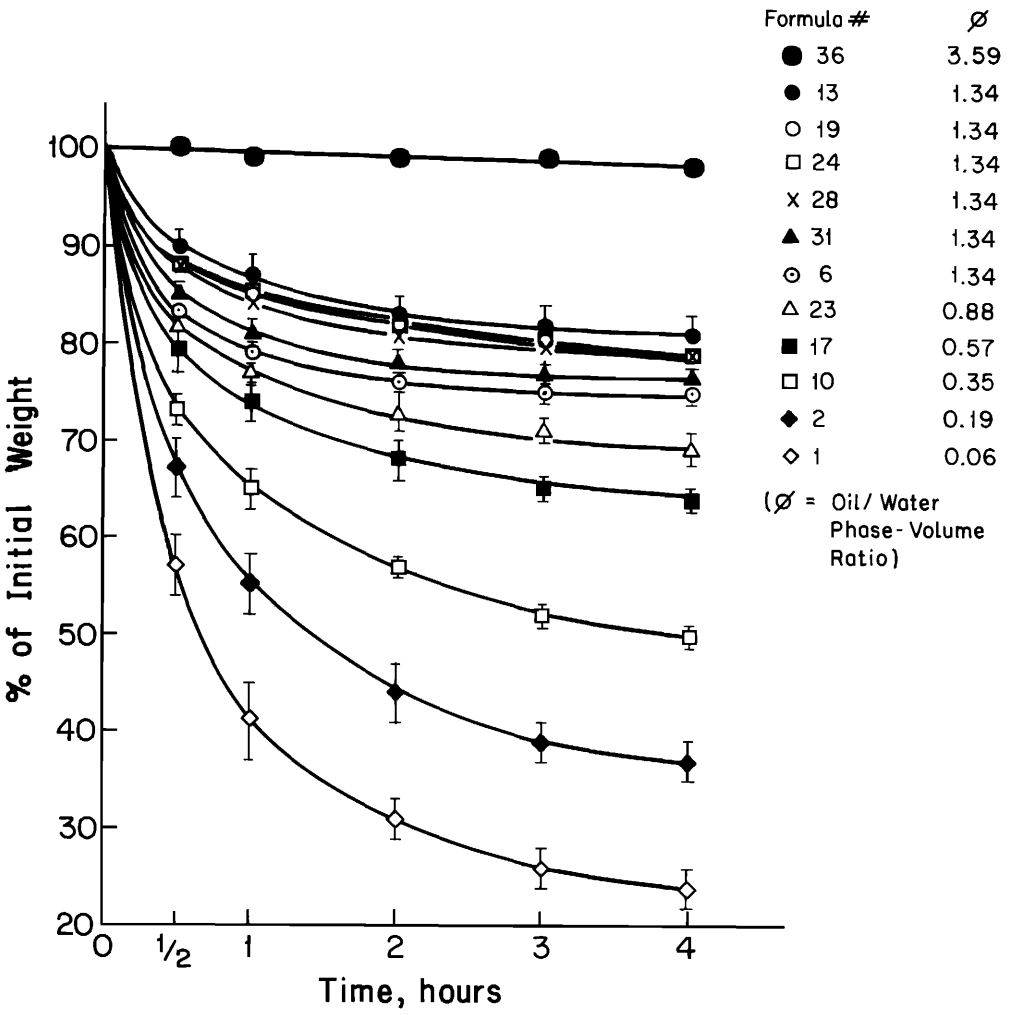

Figure 3. The percentage of initial weights of the 12 selected hydrocortisone 17-valerate 0.2% o/w emulsions in four hrs. The bar indicates the standard error of the means (n = 3).

always occur if the water vapor from the o/w vehicle is taken away more quickly than water can diffuse upward from the deeper layer of the skin into the stratum corneum. The situation applied to the highly volatile emulsions with low phase-volume ratio, i.e., formulas #1, #2, #10, #17, and #23. In theory, it is speculated that after losing most of their own water, the emulsions will develop a draining effect, which can lead to drying of the underlying tissue (2). However, the evaporation could also cause a corresponding rise in concentration of active drug in these highly volatile formulas, which might then increase the rate of diffusion of the active into the skin. Ideally, an optimal formula might be achieveable by adjusting its phase-volume ratio to the optimal point in which the skin permeation of the active is enhanced to a maximum level. The following study using the selected twelve formulas is an attempt to achieve this goal.

TRANSEPIDERMAL WATER LOSS DETERMINATIONS

Figure 4 presents the experimental results of transepidermal water loss (TEWL), represented in terms of water evaporation rate (WE). A similar trend is found in Figure 4 as in Figures 2 and 3, where formulas #1 and #2, which show the highest volatility or weight loss, also provided the highest TEWL in 90 min, and formula #36, which shows the least volatility or weight loss, provided the lowest TEWL at a practically constant water evaporation rate. Other formulas were between the two extremes. The TEWL trend was also found to correspond well with their o/w phase-volume ratio.

The mean of TEWL of bare skin measured as a base value prior to the emulsions determination was 7.7 ± 2.1 g/m^{2}h. It is therefore safe to assume that formulas with a TEWL value smaller than 5.6 g/m^{2}h (the lower margin of the mean value) can be plausibly considered as occlusive. Formula #36 was the most occlusive vehicle; it provided occlusivity at the time of the application. Formulas #1 and #2 did not provide any occlusivity in 90 min. Formulas #19, #24, #28, and #31 with phase-volume ratio (1.34) provided TEWL below 5.6 g/m^{2}h 30 min after application, indicating that occlusivity occurred after most of the water evaporated. The other formulas had TEWL values close to the value of the bare skin, indicating that they provided little occlusivity. Increasing the amount of mineral oil from 0% to 10% at a constant percentage of petrolatum did not materially affect the TEWL value. On the other hand, increasing the concentration of petrolatum at a constant level of mineral oil decreased the TEWL values.

The above findings, in contrast with the conventional belief that o/w emulsions provide no occlusivity, suggest that these emulsions could provide occlusivity by increasing the o/w phase-volume ratio to a level at which a hydrophobic layer could be formed after the water evaporates. The results also reveal that petrolatum provides higher occlusivity to vehicles than does mineral oil.

COSMETIC ACCEPTANCE EVALUATION

The twelve selected formulas were then tested for their cosmetic attributes according to the scoring system described in Table III.

An ANOVA F test was conducted for individual and overall characteristics. The results show that there were significant differences among the means at the 5% level for odor, texture, spreadability, and greasiness, but not for homogeneity. A multiple comparison method, Tukey's HSD test, was further performed to establish which means were different. The results suggest that formulas #31 and #36 were the least cosmetically acceptable, and the others were similar in acceptance.

IN VITRO HUMAN SKIN PERMEATION STUDIES

In vitro human skin permeation studies were conducted on the twelve selected formulas. Figure 5 represents the skin permeation profiles of formulation in group 1, while Figure 6 represents these in group 2. The permeability constants calculated according to equation (2) are listed in Table IV. Large variation occurred in some studies, presumably due to unidentified factors, such as individual skin variations, skin site variations, and uncontrolled experimental errors. However, a general trend can still be seen from the

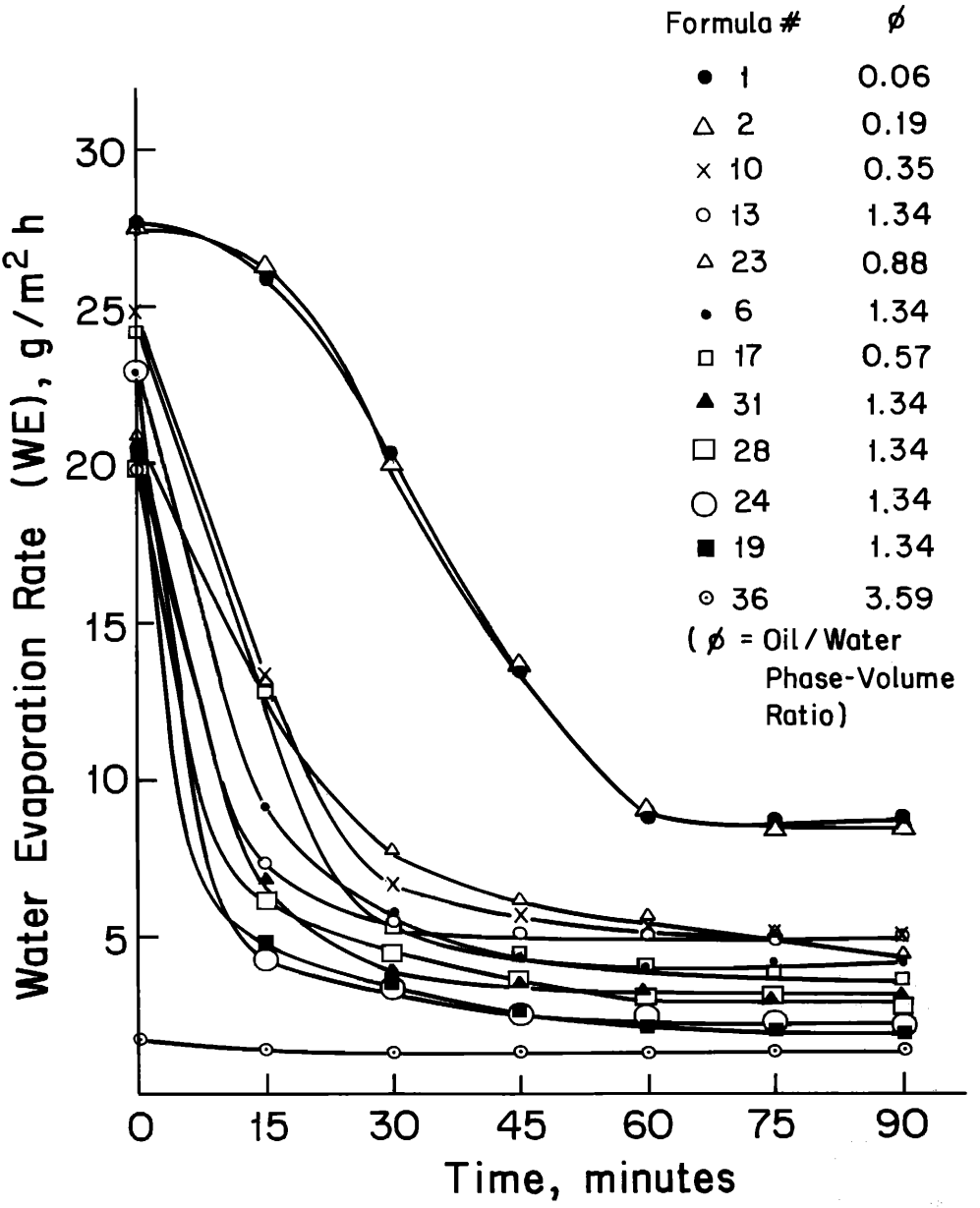

Figure 4. *In vivo* transepidermal water loss of the twelve selected hydrocortisone 17-valerate 0.2% o/w emulsions at ambient conditions (23 ± 2°C, 40 ± 5% RH).

resulting data as shown in Figures 7 and 8. Figure 7 depicts the relationship between the permeability constant of formulas and a range of phase-volume ratios from 0.19 to 1.34. A positive linear trend found from the profile suggested that the permeability rate of HCV increased as the phase-volume ratio (ϕ) was increased. Formula #28 (ϕ = 1.34) has a permeability constant (K_p) of 5.71×10^{-5} cm/hr, which is 2.4 times

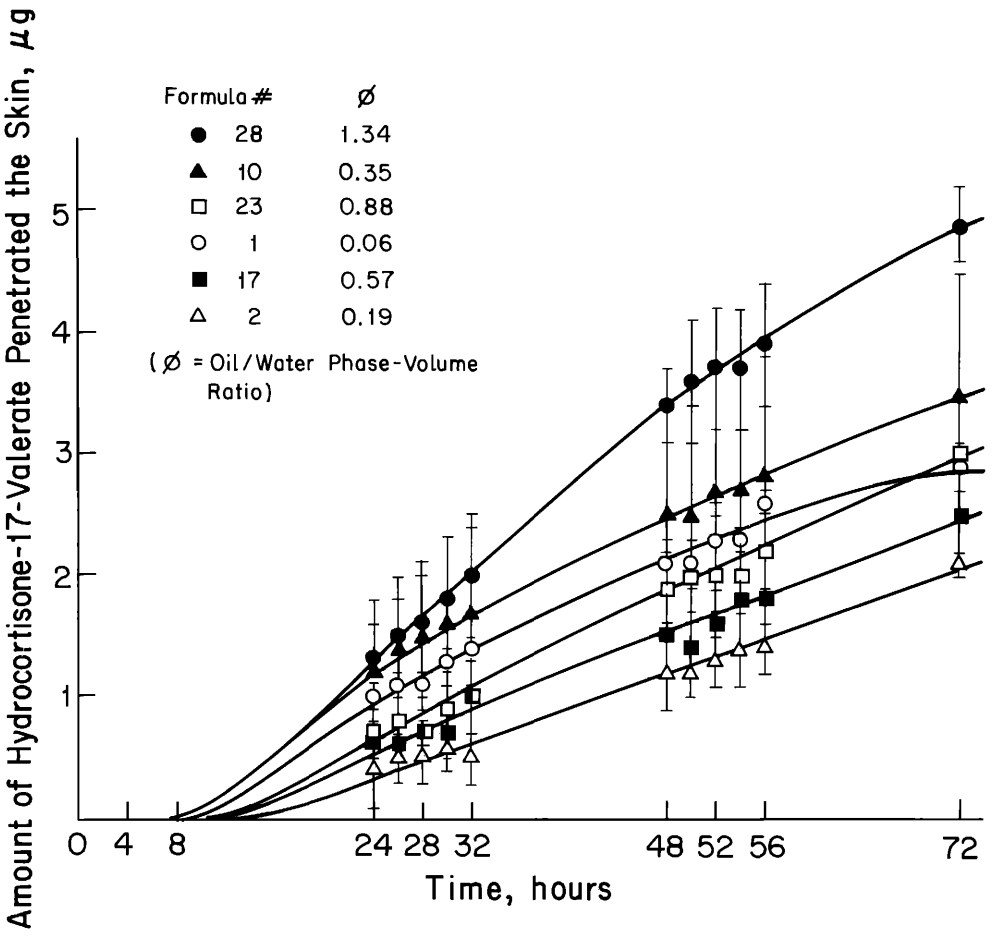

Figure 5. *In vitro* human skin permeation profiles of the selected hydrocortisone 17-valerate 0.2% emulsions with ϕ ranging from 0.19 to 1.34. The bar indicates the standard error of the means (n = 3).

higher than that of formula #2 (ϕ = 0.19). Figure 8 demonstrates the relationship of the permeability constants of formulas with a constant phase-volume ratio (ϕ = 1.34) but different % levels of petrolatum. A positive linear trend suggested that the permeability constants of HCV increase as the concentration of petrolatum increases. This was not the case for mineral oil, where the permeability constants of HCV did not increase as the concentration of mineral oil increased. Formulas # 1 and #36, the two extremes, both showed low permeability rates as compared to other formulas. The drying effect due to the high volatility was considered to result in the low permeability rate of formula #1 (2). High viscosity with low driving force in the high petrolatum vehicle, which might cause the HCV to have a tendency to stay in the hydrophobic phase, was presumably the cause of the low permeability of formula #36.

IN VIVO VASOCONSTRICTOR ACTIVITY DETERMINATION

All the experimental results from the above studies suggested that formula #28, which contains 28.9% water, 40% petrolatum, and 10% mineral oil, is the most desirable

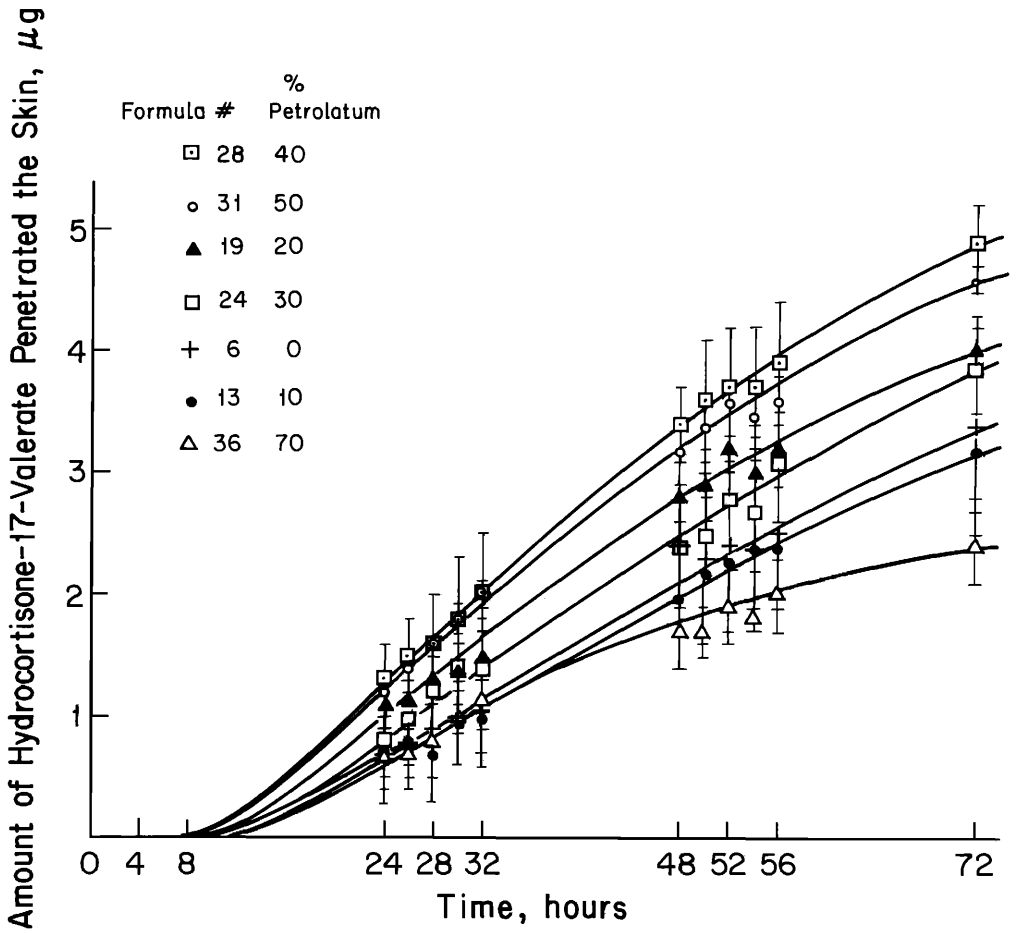

Figure 6. *In vitro* human skin permeation profiles of the selected hydrocortisone 17-valerate 0.2% emulsions with $\phi = 1.34$. The bar indicates the standard error of the means (n = 3).

choice for hydrocortisone 17-valerate. Formula #28 was then included in the comparative efficacy and safety evaluations against some marketed corticosteroid products.

The degree of vasoconstriction induced by HCV 0.2% in formula #28 and a marketed cream, relative to that of other corticosteroid products, was evaluated by Sefton *et al.* (1) as shown in Table V. The results indicated that 0.2% HCV in both formula #28 and cream is associated with equal or greater vasoconstrictor responses than are other corticosteroid preparations of intermediate or moderate potency corticosteroids, including formulations of triamcinolone acetonide 0.1% cream, fluocinonide 0.05% cream and ointment, and betamethasone 17-valerate 0.1% cream. It was also noted that formula #28 induces statistically greater vasoconstriction (by Duncan's procedure) than does HCV cream at 0.2% level.

In order to examine the relationship between vasoconstrictor activity and skin permeation rate, an additional *in vitro* skin permeation study on the marketed HCV 0.2% cream was performed. According to the formulation information (7,8), the HCV 0.2% cream has a phase-volume ratio of 0.61. The resulting data from the skin permeation

Table IV
Permeability Constants (K_p) of the Selected 12 Hydrocortisone 17-Valerate 0.2% o/w Emulsions (n = 3)

Formula no.	K_p, cm/hr	ϕ
1	3.20×10^{-5} (3.97×10^{-6})*	0.06
2	2.40×10^{-5} (8.66×10^{-7})	0.19
6	4.34×10^{-5} (5.54×10^{-6})	1.34
10	3.57×10^{-5} (9.01×10^{-6})	0.35
13	4.14×10^{-5} (1.85×10^{-6})	1.34
17	3.08×10^{-5} (4.89×10^{-6})	0.57
19	5.11×10^{-5} (3.03×10^{-7})	1.34
23	3.56×10^{-5} (7.10×10^{-6})	0.88
24	4.78×10^{-5} (3.85×10^{-6})	1.34
28	5.71×10^{-5} (1.05×10^{-7})	1.34
31	5.46×10^{-5} (8.31×10^{-7})	1.34
36	3.10×10^{-5} (3.65×10^{-6})	3.59

* Standard error of the means (n = 3).

study indicate that the permeability constant 3.50×10^{-5} cm/hr (s.e. = 5.10×10^{-7} cm/hr) of the marketed HCV 0.2% cream was 0.6 times that of 5.71×10^{-5} cm/hr (s.e. = 1.05×10^{-7} cm/hr) of formula #28. Correspondence between the *in vitro* skin permeation rate and the *in vivo* vasoconstrictor activity is indicated in this study. It is therefore reasonable to assume that the enhanced vasoconstrictor activity of formula #28 can be attributed to its higher occlusivity.

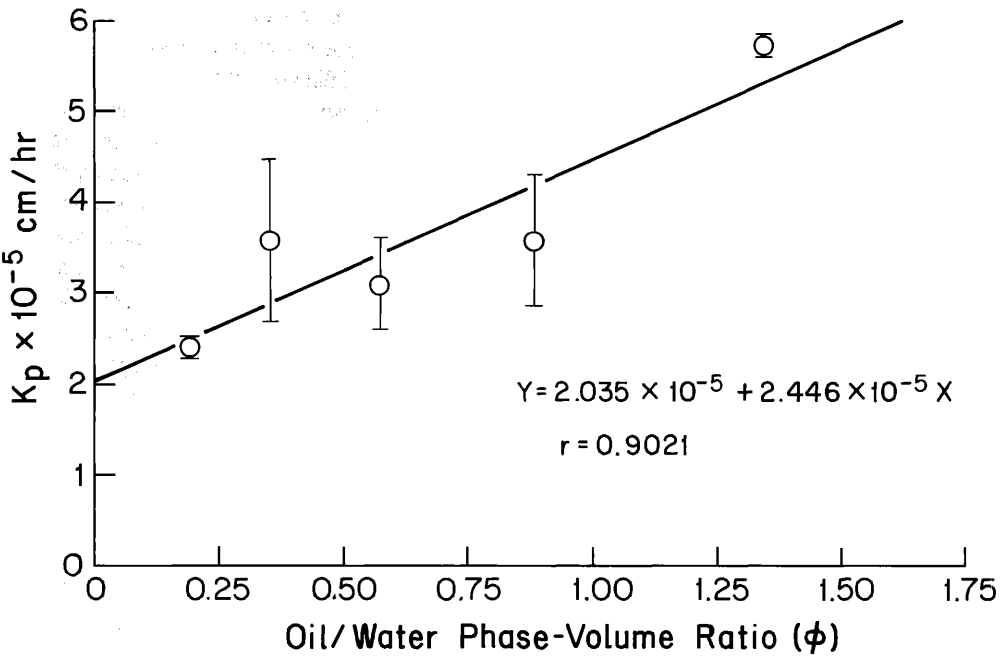

Figure 7. Correlation of the permeability constants and phase-volume ratios (ϕ) of the selected o/w emulsions. The bar indicates the standard error of the means (n = 3).

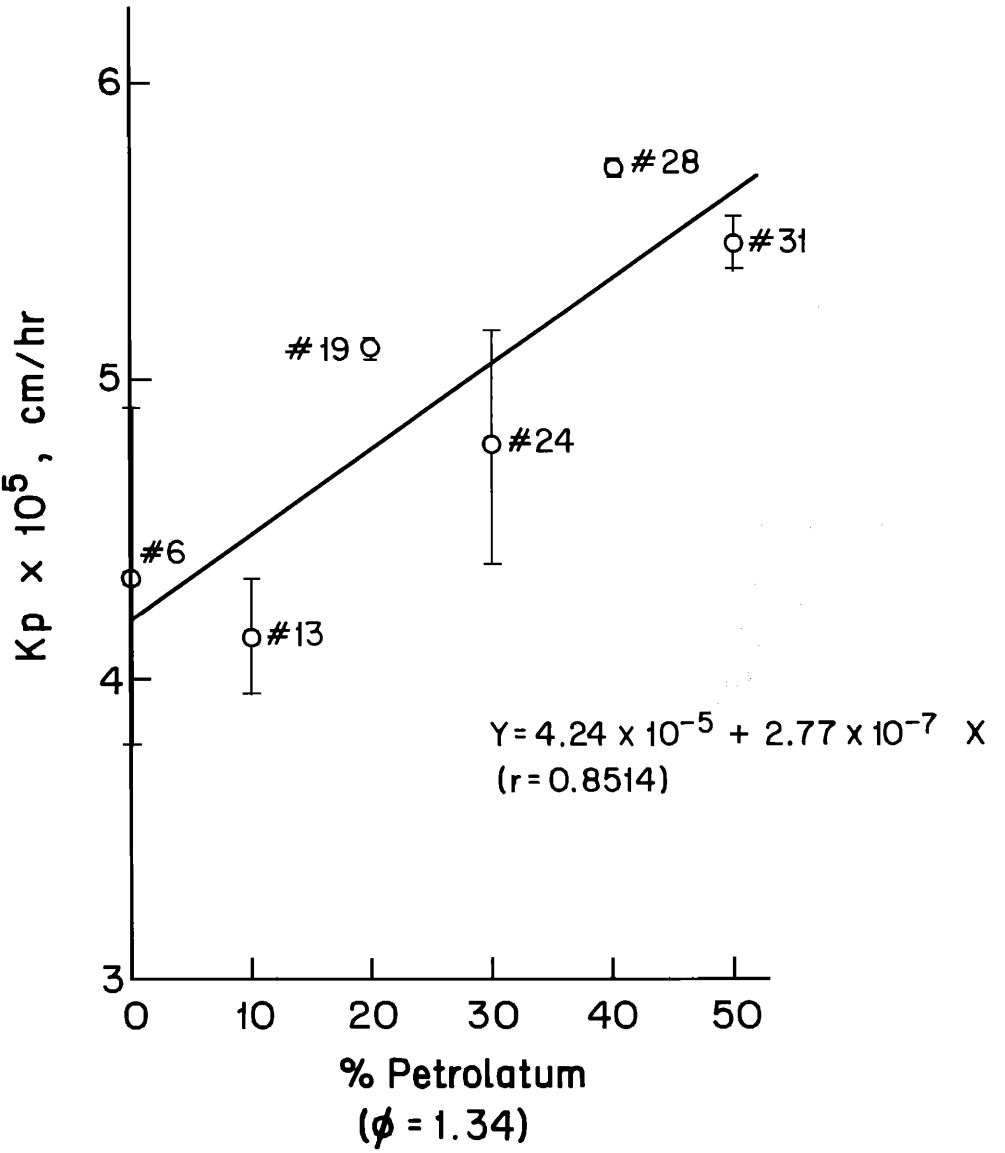

Figure 8. Linear correlation of permeability constants and % of petrolatum of the selected o/w emulsions with $\phi = 1.34$. The bar indicates the standard error of the means (n = 3).

CONCLUSIONS

In conclusion, the findings of this study suggest that the occlusivity of an o/w emulsion system can be enhanced by adjusting its o/w phase-volume ratio with petrolatum. This enhanced occlusivity will, in turn, promote the *in vitro* human skin permeation rate. However, occlusivity provided by too high a concentration of petrolatum did not enhance the permeation of HCV. This study shows that petrolatum, not mineral oil, is the key ingredient providing the occlusivity of the o/w emulsion system. *In vivo* vaso-

Table V
Vasoconstrictor Activity of Various Corticosteroid Creams and Ointments in Relation to That of Hydrocortisone 17-Valerate 0.2% Cream (assigned the value of 1.00)

Formulation	Mean activity (n = 24)
Fluocinonide 0.05% ointment (Lidex, Syntex Lab Inc., Palo Alto, CA)	1.51[a]*
Fluocinonide 0.05% cream (Lidex, Syntex Lab Inc., Palo Alto, CA)	1.27[b]
Desoximetasone 0.25% cream (Topicort, Hoechst-Roussel Pharm. Inc., Somerville, NJ)	1.22[c]
Hydrocortisone Valerate 0.2% emulsion (Formula #28, later designated HCV 0.2% ointment) (Westcort, Westwood Pharm. Inc., Buffalo, NY)	1.11[d]
Desoximetasone 0.05% cream (Topicort, Hoechst-Roussel Pharm. Inc., Somerville, NJ)	1.07[d]
Hydrocortisone Valerate 0.2% cream (Westcort, Westwood Pharm. Inc., Buffalo, NY)	1.00[e]
Betamethasone Valerate 0.1% cream (Valisone, Schering Corp., Kenilworth, NJ)	0.47[f]
Betamethasone Valerate 0.01% cream (Valisone, Schering Corp., Kenilworth, NJ)	0.46[f]
Triamcinolone Acetonide 0.1% cream (Kenalog, E. R. Squibb & Sons Inc., Princeton, NJ)	0.32[g]

* For each assay, means identified with different letters (i.e., a–g) are significantly different ($P \leq 0.05$ for adjacent means) from each other by Duncan's procedure. Different sets of formulations were tested in each assay. (Courtesy of Sefton *et al.* (1).)

constrictor activity studies further support the *in vitro* findings, where formula #28 has a higher skin permeation rate as well as a higher vasoconstrictor activity than that of the HCV 0.2% cream, which could be due to the occlusivity of the vehicle.

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