# Optimizing Dermatological Formulations of Retinoic Acid

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**Abstract** □ Formulations of retinoic acid for the topical treatment of ichthyosiform and seborrheic dermatoses were developed. They were evaluated for their skin irritation potential on rabbits, as a measure of pharmacological "activity," by studying erythema and scab-formation times. Different types of vehicles and varying concentrations of retinoic acid were studied. It was found that relatively high concentrations of retinoic acid (0.1–0.2%) were necessary in lipophilic vehicles (in which retinoic acid is relatively insoluble) to achieve a desirable irritation level. Such formulations should be useful for treating ichthyosiform dermatoses. Conversely, it was found that relatively low concentrations of retinoic acid (0.001–0.050%) were preferable in hydrophilic vehicles (in which retinoic acid is relatively soluble) to achieve a desirable irritation level. Such formulations should be useful for treating seborrheic dermatoses.

**Keyphrases** □ Retinoic acid—development of topical formulations for treatment of ichthyosiform and seborrheic dermatoses □ Topical formulations, retinoic acid—developed for treatment of ichthyosiform and seborrheic dermatoses □ Dermatology—retinoic acid formulations developed for treatment of ichthyosiform and seborrheic dermatoses □ Ichthyosis, treatment—development of retinoic acid formulations □ Seborrhea, treatment—development of retinoic acid formulations

The application of vitamin A acid or retinoic acid to therapy of topical diseases was first reported by Stüttgen in 1962 (1). He observed irritation in which vasodilation and slight perivascular infiltration were seen and in which keratolysis of the epidermis occurred with no observable effects on the dermis. He reported some success in treating ichthyosis vulgaris, senile hyperkeratosis, and acanthosis, using lipophilic ointments containing 0.1–1.0% retinoic acid. He observed minimal pharmacological activity without irritation in ichthyosis, using an ointment containing 0.01% retinoic acid. He found neither activity nor irritation using a 0.01% alcoholic solution.

Subsequently, much new information has been added. Possible effectiveness in Darier's disease (2, 3), keratoacanthomas (4), acne (2, 5–8), psoriasis (2, 9), congenital ichthyosiform erythroderma (2, 10), zosteriform keratosis (11), lamellar ichthyosis (12), and wound healing (13) has been reported. Of special interest are the reports on the treatment of seborrheic conditions such as acne, in which hydrophilic vehicles are preferred, and of ichthyosiform conditions, in which hydrophobic vehicles are preferred.

From the early reports that discuss vehicles and retinoic acid concentration in relation to irritation, it was hypothesized that irritation is probably directly related to retinoic acid activity and that a desirable "activity" level would be mild erythema with little or no scab formation. Thus, screening of vehicles and evaluation of concentration effects could be determined effectively in animal skin irritation tests. Ichthyosiform dermatoses can best be treated with lipophilic vehicles that partially occlude the area, render the skin more pliable, and offer physical protection. The skin barrier is not intact due to the disease; to prevent rapid penetration and to achieve a sustained local effect, retinoic acid should be relatively insoluble in the vehicle. However, seborrheic dermatoses (especially acne) can best be treated with hydrophilic vehicles that tend to remove excess sebum, impart a desirable drying effect, and enhance retinoic acid absorption. This can be accomplished using a vehicle that penetrates well and also dissolves retinoic acid well. Thus, two different types of vehicle are required for broad clinical evaluation.

As a result of these considerations, a series of experiments was planned. The objectives were: (*a*) to determine the purity and stability of retinoic acid and its solubility in various materials that could be used in dermatological formulations; (*b*) to choose the materials in which the retinoic acid showed greater solubility for possible use as hydrophilic vehicles and those in which it showed lesser solubility for use as hydrophobic vehicles; (*c*) to formulate o/w and w/o test vehicles; and (*d*) to carry out a pharmacological screening study to assist in selecting optimal formulations containing sufficient retinoic acid to produce a desirable, mild irritation without undesirable edema and scab formation. The solvents and the formulations used are identified below.

## EXPERIMENTAL

**Chemicals**—All of the commercially available isomers of retinoic acid were investigated, and all-*trans*-retinoic acid[1] was chosen for these studies. This is the most readily available form and has been used in some reported clinical studies. Of course, the activity of physical forms of all-*trans*-retinoic acid other than that used and of other isomers and combinations of isomers may be different than that chosen, so these results will not necessarily be representative of all forms.

The purity of all-*trans*-retinoic acid was verified by UV, IR, differential scanning calorimetry, TLC, and column chromatography, and good evidence of at least 95% purity was obtained.

Sherman (14) demonstrated unusually good stability for an all-*trans*-retinoic acid solution in petroleum ether subjected to UV light. The sclution also showed good stability in various other organic solvents when stored in the dark at room temperature for up to 3 months. These results have been verified in the authors' laboratory for some solvents and for the formulations reported here.

**Solubility**—Retinoic acid solubility in potential ointment vehicles was determined, and it was found that higher molecular weight liquid alkanols and polyethylene glycol ethers of these alkanols were relatively good solvents, but that fatty esters, cholesterol esters, and hydrocarbons were relatively poor. Representative room temperature solubilities (percent w/w) are as follows: lauryl alcohol[2], 2.5; 2-octanol, 1.8; ethoxylated lauryl alcohol[3], 1.5; polyethylene glycol 400, 0.5; almond oil, 0.2; isopropanol, isopropyl myristate, and acetylated cholesterols[4], 0.2; and mineral oil, 0.008. A supersaturated solution (0.04 at room temperature) was obtained by incorporating retinoic acid in liquid petrolatum at 58°.

**Formulation**—Using this solubility information, an o/w cream (Formula A) in which retinoic acid is relatively soluble was formu-

[1] Eastman Kodak Co.
[2] Loral 5, Du Pont.
[3] Brij 30, Atlas.
[4] Acetulan, American Cholesterol Products, Inc.

**Table I**—Identification of Formulas

| Treatment | Percent Retinoic Acid | Description of Vehicle |
|---|---|---|
| 1 | 0.2000 | Formula B[a] (w/o, mineral oil) |
| 2 | 0.2000 | Formula B[b] (w/o, mineral oil) |
| 3 | 0.2000 | Formula C[a] (w/o, almond oil) |
| 4 | 0.2000 | Formula C[b] (w/o, almond oil) |
| 5 | 0.2000 | Formula A (o/w, lauryl alcohol) |
| 6 | 0.0500 | Formula A (o/w, lauryl alcohol) |
| 7 | 0.0100 | Formula A (o/w, lauryl alcohol) |
| 8 | 0.0500 | Mineral oil |
| 9 | 0.2000 | Mineral oil |
| 10 | 0.0100 | Almond oil |
| 11 | 0.0500 | Almond oil |
| 12 | 0.2000 | Almond oil |
| 13 | 0.0100 | Lauryl alcohol |
| 14 | 0.0500 | Lauryl alcohol |
| 15 | 0.2000 | Lauryl alcohol |
| 16 | 0.0500 | Formula D (o/w, hexadecyl alcohol) |
| 17 | 0.0000 | Formula A (o/w, lauryl alcohol) |
| 18 | 0.0001 | Formula A (o/w, lauryl alcohol) |
| 19 | 0.0010 | Formula A (o/w, lauryl alcohol) |

[a] Retinoic acid incorporated cold. [b] Retinoic acid incorporated hot.

lated. It contained the following ingredients (percent w/w): laury[1] alcohol, 2; ethoxylated lauryl alcohol, 3; propylene glycol, 5; stearyl alcohol, 4; ethoxylated stearic acid[5], 3; glyceryl monostearate, 4; silicone 500 CST[6], 2; and water *qs*. The first three ingredients, based upon the solubility data, apparently contributed most of the solubility characteristics of the cream. For the w/o ointment (Formula B), in which retinoic acid is relatively insoluble, cold cream USP XVII containing spermaceti, white wax, mineral oil, sodium borate, and water were used. None of these ingredients appeared to contribute appreciably to retinoic acid solubility. A w/o cold cream formula, substituting almond oil for mineral oil, was also prepared (Formula C). The ointments were milled so that retinoic acid crystals were reduced to a mean size of about 50 $\mu$. An alternate o/w cream (Formula D), similar to Formula A but containing 5% hexadecyl alcohols[7] instead of lauryl alcohol and ethoxylated lauryl alcohol, was also evaluated. The chemical and physical stabilities of these formulations were determined to be adequate for testing purposes. The formulas are identified in Table I.

**Pharmacological Screening**—Preliminary work indicated that the albino rabbit would be an acceptable model. For Experiment 1, New Zealand White male rabbits were selected and were equilibrated with plastic collars. It was planned to evaluate 16 formulations (Table I), and 16 rabbits were randomly selected for testing from a larger group. The experimental design was a Youden "square," having six rows and 16 columns, with rows representing positions on each animal's back and columns representing individual rabbits. A unique subset of six of the 16 formulations was to be tested on the six sites on each rabbit's back. This Youden square design has the characteristic that every formulation is tested an equal number of times (six), and every formulation appears together with every other one an equal number of times (twice). The subsequent statistical analysis of the data, described below, ensured that treatment means would be adjusted to isolate the influences of position (if any) and animal differences, so that treatments could be compared in an unbiased fashion.

The dorsal-lateral hair was removed from the backs of the 16 animals by careful clipping on the day before the test began. Tattoos were applied to designate test areas on each back. A template providing six 3.8 × 7.6-cm. (1.5 × 3.0-in.) sites, with 3.8 cm. (1.5 in.) or more between each, was used to locate the six positions. On the 1st day of test, a 100-mg. quantity of test ointment or liquid was applied to each site, using a smooth polyethylene spatula to distribute the test material uniformly. The order of treating the 16 sites on each rabbit's back was randomized, and for each subsequent application, new randomizations were used. Treatments 1–16 were used (Table I).

[5] Myrj 52, Atlas.
[6] Dow.
[7] Enjay.

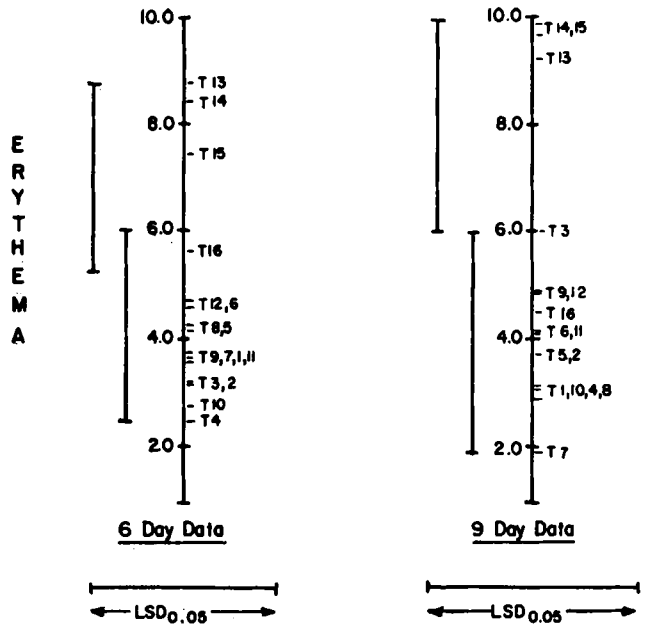


**Figure 1**—*Tukey multiple-comparison tests for all two-treatment erythema comparisons of the first 16 treatments listed in Table I. Mean scores are arranged in rank order. $LSD_{0.05}$ values are plotted on the same scale.*

The six sites on each back were treated daily for 10 days. Prior to each daily application, each treated area was carefully observed, again in a random order and with the observer having no knowledge of the identity of the formulation used at each site. One observer scored each site for erythema each day in this way, using a scale of 1–12 (1 = "no erythema," 12 = "maximum erythemal response"). Edema was scored similarly and separately by the same observer.

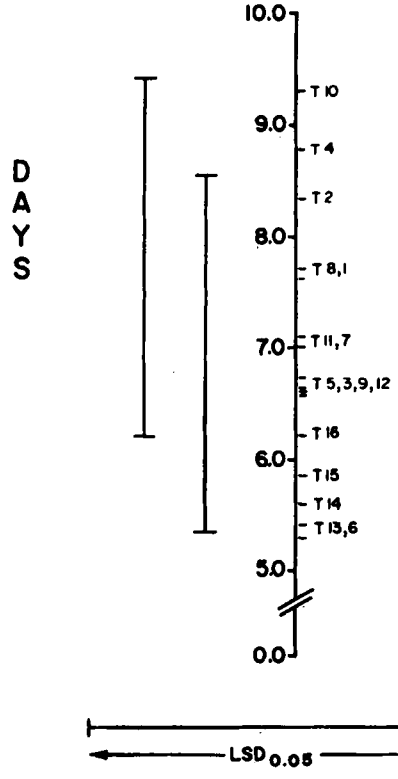


**Figure 2**—*Tukey multiple-comparison tests for all two-treatment scab formation time comparisons of the first 16 treatments listed in Table I. Mean scores are arranged in rank order. $LSD_{0.05}$ values are plotted on the same scale.*

Table II—Mean Erythema Scores for Formula A (o/w, Lauryl Alcohol)

| Treatment | Retinoic Acid Concentration % | Retinoic Acid Concentration p.p.m. | Means[a] Day 3 | Means[a] Day 6 | Means[a] Day 9 |
|---|---|---|---|---|---|
| 17 | 0.0000 | 0 | 1.81 | 1.88 | 1.84 |
| 18 | 0.0001 | 1 | 1.91 | 1.84 | 1.78 |
| 19 | 0.0010 | 10 | 2.56 | 2.31 | 2.00 |
| 7 | 0.0100 | 100 | 3.06 | 2.75 | 2.63 |

[a] Lowest score (1) = no erythema and highest score (5) = mild erythema.

Table III—Significance Levels (Tukey Tests) for Erythema Scores in Table II

| Formula A Concentrations Compared, p.p.m. | Significance Level ($p < 0.10$) Day 3 | Day 6 | Day 9 |
|---|---|---|---|
| (0)—(1) | n.s. | n.s. | n.s. |
| (0)—(10) | <0.01 | <0.01 | n.s |
| (0)—(100) | <0.01 | <0.01 | <0.05 |
| (1)—(10) | <0.05 | <0.01 | n.s. |
| (1)—(100) | <0.01 | <0.01 | <0.01 |
| (10)—(100) | n.s | <0.05 | <0.10 |

Eschar formation was checked daily for 10 days and recorded so that scabbing formation time could be determined by adopting the convention that there was scab if it was observed in two successive days, in which case the elapsed time to formation was taken as that on the first of the 2 days. Independent statistical analyses of the erythema scores obtained on Days 3, 6, and 9 were done. These three intervals were specified *a priori*. Edema scores did not appear to contribute much information and were not analyzed. Figure 1 shows the results of the comparison of means of erythema score data using computed least-significant difference values[8] for all treatments on Days 6 and 9. Figure 2 shows the results of the comparison of means of scabbing time data using computed least-significant difference values for all treatments.

Experiment 2 was done in a similar manner, but the design provided for the use of all treatments on each animal. In this experiment, four concentrations of Formula A were studied (0, 1, 10, and 100 p.p.m.), and only four sites were used on each rabbit. Since the erythemal scores were expected to occur predominantly at the low end of the original 12-point scale and the observer was sufficiently experienced to score smaller differences, the precision of the measurement was increased by a fourfold expansion of the lower portion of the scale used previously (between 1 and 5). Edema and scabbing time were not considered important to this experiment and were not reported. The same observer scored the results in both experiments. A 4 × 4 Latin square design was used with all four levels of retinoic acid applied to each animal. The Latin square was replicated once; therefore, eight animals were used. The formulas, Treatments 7, 17, 18, and 19, are reported in Table I. The mean erythema scores are reported in Table II. Tukey HSD tests for all possible combinations are reported in Table III.

## RESULTS AND DISCUSSION

The data from Experiment 1 were analyzed after replacing missing values by least squares (there were four missing values). Then a residual analysis was done to check the effectiveness of the randomizations and to test the adherence of the data to the assumptions underlying the analysis. Treatment means adjusted for block effects were then computed, and an independent analysis of variance was done on the data from each of the 3 days chosen for analysis. There was a significant effect ($p < 0.05$) for treatments on Day 3 and a highly significant effect ($p < 0.01$) on Days 6 and 9. Scab formation time data were analyzed similarly, showing a highly significant effect for treatments ($p < 0.001$) and a significant effect for positions ($p < 0.05$).

Tukey multiple-comparison tests were done on all two-treatment comparisons for erythema for Days 6 and 9 and are shown in Fig. 1. The means of erythema scores are arranged in rank order, with the Tukey least-significant difference ($p = 0.05$) plotted alongside them on the same scale.

These comparisons show that Treatments 13, 14, and 15 were highest of all treatments on the irritation scale for Experiment 1. Since they contained very different quantities of retinoic acid but used lauryl alcohol exclusively as the vehicle, the data suggest that the alcohol contributed strongly to the irritation observed. In view of the literature, however, it was believed that retinoic acid itself is an irritant; therefore, it was hypothesized that a strong irritating effect of lauryl alcohol was masking differences in irritation due to retinoic acid concentration. The results of Experiment 2 tended to verify this hypothesis.

The poor solvents that were tested (mineral oil and almond oil) either as retinoic acid suspensions or as ointments containing 0.2–0.01% retinoic acid appeared predominantly at the low end of the irritation scale, as desired.

As mentioned, the edema scores were not sufficiently sensitive to give much additional information and are not reported.

Figure 2 presents the means of scabbing times arranged in rank order, with the slowest scabbing time being highest in the order, and the Tukey least-significant difference plotted alongside them on the same scale. These results reinforce the observation that the poor solvents gave low erythema scores.

A Spearman Rank Correlation test, done to investigate the relationship between erythema scores and scab formation time on each of the 3 days, showed rank correlation coefficients of −0.85 and −0.79 for Days 6 and 9, respectively, significant at $p < 0.01$ in both cases. These results show that either response is a reasonably good predictor of the other.

Experiment 2 was designed to investigate the dose–response effect of retinoic acid at lower concentrations (0, 1, 10, and 100 p.p.m.) in Formula A. Residual analyses were carried out, and conventional analyses of variance for Latin squares were done separately for Days 3, 6, and 9 for erythema scores only. Neither edema scores nor scabbing time observations were useful measurements at these low irritation levels. The effect of concentration was strongly significant in all cases—*viz.*, on Days 3 and 6 ($p < 0.001$) and on Day 9 ($p < 0.01$). The mean erythema scores are reported in Table II. Following the ANOVA's, Tukey least-significant difference tests were done for all pairs of concentrations for each of the 3 days. The results are shown in Table III. The table indicates that the concentrations chosen were on an effective portion of the dose–response curve and that a significant difference could be found in comparing all possible combinations except the lowest (0.0001), which was not different from zero.

By assuming that irritation does reflect retinoic acid activity, then the choice of the vehicle and the availability of retinoic acid in that vehicle must be optimized for the intended drug use.

It was concluded that, when extending these results to human clinical work, it would be advisable to treat ichthyosiform dermatoses with w/o emulsions in which retinoic acid is relatively insoluble and preferably at concentrations of 0.10% or more. For treating acneiform or seborrheic dermatoses, the preferable form would be o/w emulsions in which the solubility of retinoic acid is relatively high and preferably at concentrations of 0.05% or less.

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[8] Tukey HSD test.

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# Alterations in Absorption of Dicumarol by Various Excipient Materials

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**Abstract** □ A plasma level study was conducted in dogs to determine the effects of various excipient materials on the absorption of dicumarol. The drug was combined with the excipients by an equilibration process and administered orally. Plasma concentrations of dicumarol after administration with excipients were compared to control levels produced by the drug given alone. Significant differences in the plasma levels of dicumarol were observed with six of the 10 excipients used in the study. Significantly higher plasma levels (up to 180% of control values) were observed when dicumarol was administered with magnesium oxide or hydroxide. This effect may be due to chelate formation because the magnesium chelate of dicumarol produced higher plasma levels of dicumarol than the drug administered alone. Dicumarol administration with talc, colloidal magnesium aluminum silicate, aluminum hydroxide, or starch resulted in significantly lower plasma levels of the drug. It is suggested that these types of interactions may be an explanation for differences in the bioavailability of dicumarol from different dosage formulations.

**Keyphrases** □ Dicumarol absorption—effect of various excipients on plasma levels, dogs □ Absorption, dicumarol—effect of various excipients on plasma levels, dogs □ Plasma levels, dicumarol—effect of various excipients, dogs □ Excipient effect—dicumarol absorption, plasma levels, dogs □ Bioavailability, dicumarol—effect of various excipients on plasma levels, dogs □ Magnesium oxide, hydroxide excipients—effect on dicumarol absorption, dogs

Substantial evidence is available to show that the absorption characteristics and, ultimately, the therapeutic performance of a drug can be significantly altered by changes in the materials and methods used in its formulation. Wagner (1) recently provided a review of studies involving the determination of generic equivalency or inequivalency of different commercial brands of a single drug. Ten of the 12 drugs studied showed significant differences in the bioavailability of drug when two or more commercial brands of the same drug were compared.

Lach and his coworkers (2–4), through diffuse reflectance spectroscopic studies, demonstrated that some adjuvants used in product formulation interact with certain drugs to form complexes. These combinations exhibited markedly different diffuse reflectance spectroscopic characteristics compared to those of the drug alone. The oral anticoagulant dicumarol was shown to interact with various organic and inorganic formulation materials, and it was suggested that these types of interactions may alter the bioavailability of the drug (3). Dicumarol has been shown to be poorly absorbed (5), and its bioavailability may be particularly susceptible to formulation changes. Losinski (6) reported that formulation changes involving the addition or reduction of inert fillers in tablets of dicumarol resulted in significant therapeutic variations.

The purpose of this study was to investigate whether the absorption of dicumarol could be altered by administration of the drug in combination with various formulation materials. The characteristics of its absorption and elimination, coupled with a pharmacological response dependent on plasma concentration, permitted the use of dicumarol to show that drug–excipient interactions may be an explanation for differences in therapeutic efficacy among different dosage formulations.

## EXPERIMENTAL

**Materials**—Dicumarol[1] was recrystallized from dioxane (m.p. 288–289°). Other materials used were: magnesium oxide, heavy[2]; magnesium hydroxide, reagent grade[2]; magnesium stearate USP; dibasic calcium phosphate NF, hydrous[2]; aluminum hydroxide powder[2]; colloidal magnesium aluminum silicate[4]; silicic acid[5]; starch USP; talc USP; polyvinylpyrrolidone[6]; and polysorbate 80[7].

Elemental analysis of the magnesium chelate of sodium dicumarol[8] showed it to have the form $(dicumarol\text{-}Na)_2Mg$. A method for the preparation of the chelate was reported previously (4).

**Animals**—Eight healthy mongrel dogs of either sex, weighing 10–21 kg., were utilized in the study. The animals were housed in stainless steel cages and fed dog chow[9] between 8 a.m. and noon daily, unless specified otherwise.

**Dicumarol–Excipient Mixtures**—Dicumarol was mixed with an excipient in two different weight to weight ratios—6 dicumarol:94 excipient and 1 dicumarol:1 excipient. Dicumarol was dissolved in chloroform to make a solution containing 0.3 g. in 50 ml. An appro-

[1] K and K Laboratories.
[2] Mallinckrodt.
[3] Matheson, Coleman and Bell.
[4] Veegum, regular, Vanderbilt.
[5] Fisher.
[6] Antara Chemicals.
[7] Tween 80, Atlas.
[8] A gift from Dr. L. Bighley, College of Pharmacy, University of Iowa.
[9] Purina.