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pH Effects on Salicylate Absorption from Hydrophilic Ointment

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Abstract □ Hydrophilic ointment formulations containing salicylic acid were prepared at different pH levels by inclusion of phosphate buffers. Percutaneous salicylic acid absorption was studied by measuring salicylate blood levels in rabbits at 1.5-hr. intervals from 1.5 to 7.5 hr. after ointment application. The effect of 15% dimethyl sulfoxide in the various formulations was also determined. Formulations without dimethyl sulfoxide produced maximal blood concentrations at the highest pH level (10.78) and higher concentrations at the lowest pH (2.97) than at the intermediate ones (4.48, 6.80, 9.23). Proportionally higher concentration of undissociated species appeared to account for the increased absorption observed at the most acidic pH, while increased dissolution was probably responsible for the greater blood levels recorded at the most alkaline pH. Dimethyl sulfoxide produced a more rapid rate of salicylate absorption as well as greater peak blood levels at each pH. However, its influence on rate of absorption and on peak blood levels was less pronounced at the highest pH levels. This finding seems to indicate that the positive effect of dimethyl sulfoxide on percutaneous salicylate absorption is due in part to its ability to enhance the dissolution of salicylic acid.

Keyphrases □ Salicylate absorption—hydrophilic ointment □ pH effect—salicylate absorption from hydrophilic ointments □ Dimethyl sulfoxide effect—salicylate absorption, hydrophilic ointment □ Percutaneous absorption—salicylate in hydrophilic ointment

Experimental evidence indicates that percutaneous absorption of most drugs occurs by passive diffusion of an undissociated therapeutic entity across a lipoidal barrier (1). Characteristics of an ointment base, in particular its thermodynamic activity and included solvents, influence the rate of release from the base and the rate and quantity of percutaneous penetration and absorption. Knowledge of the specific influence of each of these factors can aid in the formulation and choice of ointment bases designed to elicit a specified rate and magnitude of percutaneous drug absorption for a particular drug administered topically.

Higuchi (2) indicated that the chief driving force for diffusion and penetration is thermodynamic activity, which for a weakly acidic drug is inversely proportional to the term 10^{pH}. Bhatia and Barber (3) found changes in the local anesthetic activity of ethyl aminobenzoate incorporated into hydrophilic ointment USP buffered at nine different pH values ranging from 3.5 to 10.0. They observed maximum pharmacologic activity at pH 6 and 7 and found a marked decrease when the pH was decreased or increased from that neutral region. Bhatia and Barber (3) attributed their results to the possibility that maximum penetration and pharmacologic activity occurred at or near the pH of rat skin. Stolar *et al.* (4) theorized that the concentration of free salicylic acid present at a pH of 6.2–6.5, in either an aqueous solution of 6.95% sodium salicylate or in a sodium salicylate cream having an aqueous dispersion medium, could not account for the measurable salicylate blood levels observed in rabbits. Blank and Gould (5) observed an increase in the absorption of sodium laurate solutions in contact with excised human skin at reduced pH. They attributed the increase in absorption at the lower pH to the formation of more undissociated lauric acid.

The use of solvents to enhance percutaneous absorption was suggested by the ease with which gases, such as nitrobenzene vapor (6), can penetrate the skin (7–10), and has been confirmed by the greater enhancement of percutaneous absorption produced by the volatile

Table I—Buffer Systems and Corresponding pH Values for Aqueous Phase

Aqueous Phase, pH	Salt or Buffer Pair	g./30 g. of Ointment	moles/30 g. of Ointment,
2.97	KH_2PO_4[a]	1.0048	0.0074
	H_3PO_4[a]	0.1096	0.0011
4.48	KH_2PO_4[a]	1.1568	0.0085
6.80	K_2HPO_4[b]	0.7403	0.0042
	KH_2PO_4[a]	0.5784	0.0042
9.23	K_2HPO_4[b]	1.4805	0.0085
10.78	$K_3PO_4 \cdot H_2O$[a]	0.4033	0.0019
	K_2HPO_4[b]	1.1496	0.0066

[a] Mallinckrodt Chemical Works, St. Louis, Mo. [b] Analytical reagent grade, Fisher Scientific Co., Pittsburgh, Pa.

Table II—Experimental Design for the Four Rabbits Tested at Each pH Level[a]

	Variables Tested among Different Animals		Variables Tested in Each Animal			
Rabbit	Replicate	Initial Condition	Trial 1	Trial 2	Trial 3	Trial 4
			Stage 1		Stage 2	
1	1	No DMSO	No	DMSO	No	DMSO
2	1	DMSO	DMSO	No	DMSO	No
3	2	No DMSO	No	DMSO	No	DMSO
4	2	DMSO	DMSO	No	DMSO	No

[a] Two variables tested among different animals (Initial Drug Condition and Replications), and, for the four trials, two variables tested in each animal (Drug Condition and Stages). "No" here refers to the absence of DMSO from the ointment base.

component of a volatile–nonvolatile topical steroid solution (11).

One nonvolatile solvent, dimethyl sulfoxide (DMSO), which has received much attention in recent years, has been found to enhance the percutaneous absorption of numerous drugs (12–18). Mechanisms proposed to explain the effect of DMSO include its reversible interaction with keratin, the extraction of lipids within the skin by disulfide interchange, the possibility of its interaction with another diffusing solute, and its inhibition of the redox polymerization of hyaluronic acid (19–22). Studies to date have indicated that DMSO is low in toxicity and causes no abnormal changes in the eyes of man, even when applied directly to the eyes (23).

The objective of this study was to determine the influence of pH on the percutaneous absorption of salicylic acid from cream-type bases, using rabbits as the test animals. In addition, the effect of DMSO, which is known to influence the absorption of salicylic acid and sodium salicylate (24), perhaps partially by altering the solubility characteristics of the drug, was investigated at different pH values. The measurement of effects of DMSO at several different pH levels may indicate the optimal combination for either maximizing or retarding the rate and magnitude of percutaneous drug absorption and can provide information on the mechanisms by which pH and DMSO both influence absorption.

MATERIALS AND METHODS

Animals—The study employed a total of 20 white, male, New Zealand rabbits with a weight range of 1.68–3.48 kg. and an average weight of 2.70 kg. They were housed in temperature- and humidity-controlled cages, and were supplied with Purina[1] rabbit chow and water *ad libitum*, except that food was withdrawn for 24 hr. prior to each ointment application.

Preparation of Ointment Base—Salicylic acid[2] particle size was reduced in a ball mill,[3] and the material was passed through an 80-mesh sieve. The powder was dried in a heated vacuum desiccator[4] at 50° for a minimum of 48 hr. Hydrophilic ointment USP XVII (25), categorized as a water-removable ointment base, was prepared and stored in tight containers in a refrigerator at a constant temperature of 9°.

Ointment bases of various pH values were prepared by substituting the combinations and amounts of salts shown in Table I for the same weight of water in the original formula. The pH was determined with a Beckman Zeromatic II pH meter[5] for the aqueous phase of each ointment base, and a calomel junction electrode[6] attachment was exchanged for the usual electrode to observe the pH of the emulsion-type bases themselves standardized against acid mantle cream[6] of pH 4.22. The values obtained with either system, base or aqueous phase, showed only very slight differences, and the aqueous phase pH value was then utilized as the more convenient value.

After each base of each pH was prepared, 10% w/w salicylic acid was incorporated into one set of bases at each pH, and 10% w/w salicylic acid plus 15% w/w DMSO[7] was incorporated into another set of bases at each pH.

Test Procedures—The rabbits were weighed, and hair was removed with an Oster animal clipper[8] from the dorsal area and the ears 24 hr. prior to the ointment application. A 5-g. sample of ointment was spread uniformly over a rectangular area, 6.35 × 12.70 cm., on the dull side of a sheet of aluminum foil. This was applied to the shaved dorsal area of the rabbit and carefully pressed down with the aid of adhesive tape and an elastic bandage. After the 7.5-hr. test period, the tape and remaining ointment were removed, and a small amount of white petrolatum was applied to prevent chapping before the rabbit was returned to its cage.

Six blood samples were taken at intervals of 1.5 hr., the first immediately before the ointment was applied (to provide the zero time value) and the last 7.5 hr. afterward. Prior to each blood collection, the animal was set into a rabbit restraining box. One ear was warmed by manual massage and the heat from a small lamp, and a clip was placed near the base of the back of the ear to block venous blood flow as soon as the marginal ear vein distended. After the vein was pierced with a lancet[9] at a point 1 cm. above the clip, 0.5 ml. of blood was withdrawn into a syringe containing 0.1 ml. of heparin,[10] 5000 u./ml. The blood and heparin mixture was added to 5.0 ml. of a bifunctional protein precipitant and color developer in a centrifuge tube and analyzed for salicylate content according to a method originally described by Trinder (26) and more recently employed by Stelzer *et al.* (24).

Experimental Design and Statistical Analysis—The 20 rabbits were divided into five groups of four each, and each group was tested with a different pH level of ointment (2.97, 4.48, 6.80, 9.23, and 10.78). The animals were tested in pairs, one rabbit with DMSO and the other with no DMSO added to the ointment. Two such pairs (replicates) were tested with each pH level. The rabbits were given four tests, with a 7-day interval between each test. Two tests were with DMSO and the other two with no DMSO added to the ointment. Table II summarizes the experimental design for the four rabbits tested with each pH level.

Since the time of peak blood level varied in different animals and under different conditions, the blood level at any one time interval or for all intervals averaged together depended partly on absorption rate. A measure of maximal blood level, independent of rate, was obtained by recording for each animal, in each test, the highest of

[1] Ralston-Purina Co., St. Louis, Mo.
[2] Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Mo.
[3] Erweka G. m. b. H., Frankfurt-am-Main, Germany.
[4] Model No. 68351, Precision Scientific Co., Chicago, Ill.
[5] Beckman Instruments, Inc., Fullerton, Calif.
[6] Dome Laboratories, West Haven, Conn., a water-miscible, aluminum acetate-buffered cream base.
[7] Supplied by Crown Zellerbach Corp., Camas, Wash.
[8] Model A-2 with size 40 head, John Oster Manufacturing Co., Milwaukee, Wis.
[9] Medipoint, Inc., Brooklyn, N. Y.
[10] Liquaemin Sodium, Organon, Inc., West Orange, N. J.

Table III—Average of Eight Salicylate Blood Level Determinations[a]

Type of Ointment	Time, hr.		pH 2.97	pH 4.48	pH 6.80	pH 9.23	pH 10.78	Mean Values, Combined pH
No DMSO	1.5		5.75	2.47	2.58	5.49	7.03	4.66
	3.0		11.56	4.98	7.77	9.49	14.49	9.66
	4.5		12.79	7.48	8.99	10.76	16.00	11.20
	6.0		13.54	9.51	9.14	11.96	15.72	11.97
	7.5		14.07	9.98	9.34	11.01	15.66	12.01
		Mean values (Combined times)	11.54	6.88	7.56	9.74	13.78	
DMSO	1.5		13.68	8.31	10.39	8.67	11.32	10.47
	3.0		19.43	12.27	15.13	16.42	17.96	16.24
	4.5		21.38	12.58	15.24	16.70	17.37	16.65
	6.0		21.12	12.73	14.23	15.86	14.66	15.72
	7.5		18.80	11.12	12.54	14.82	12.80	14.02
		Mean values (Combined times)	18.88	11.40	13.50	14.50	14.82	

[a] Expressed as milligram percent of salicylic acid, at five time intervals after application of ointment at one of five pH values with or without DMSO.

the five blood levels in the span from 1.5 to 7.5 hr. after application of the ointment. A statistical analysis of the effects of DMSO and pH was performed for these peak blood levels as well as for blood levels as a function of time.

The milligram percent blood level of salicylic acid for each rabbit, at each of the five collection times from 1.5 to 7.5 hr., was corrected for the zero time blank. The BMD 02V Analysis of Variance Program for the IBM 7090 digital computer provided the test for statistical significance (27, 28). The analysis included three variables tested among different animals (five pH levels, two initial drug conditions, and two replications), two variables comprising different ointment applications for each animal ("no DMSO" or "DMSO" conditions, and the two stages), and one variable comprising different blood samples for each ointment application (five time periods). The residual variance for testing statistical significance, as in previous studies (29–32), consisted of the pooled interactions of all appropriate variables with the randomly selected variable (two replications). Mechanical failure of the spectrophotometer caused loss of the data on blood levels for two rabbits tested at pH 2.97 in the second of their two tests with DMSO and also in the second of their two tests with no DMSO. The scores for the first test under the same conditions were used, and the degrees of freedom for the residual variance was reduced accordingly in statistical tests for variations among different ointment applications.

For variables with more than two levels (five pH values and five time periods), the appropriate orthogonal polynomial function was used in prior studies (29–32) to test the hypothesis that increasing pH or time intervals resulted in a progressive, monotonic change in scores (linear) or monotonic change in one direction to an intermediate pH or time, followed by a monotonic change in the opposite direction (quadratic).

RESULTS

Table III summarizes the effects of the experimental treatments on blood levels of salicylic acid. Each value in the table represents an average value of blood levels obtained from the four rabbits assigned to one of the five pH levels. The value for each rabbit at each of the five time periods represents the average for two tests with and two without the addition of DMSO to the ointment.

The values in the extreme right-hand column of Table III, averages for the five pH levels, are portrayed as Fig. 1. At each time interval, the addition of DMSO resulted in higher blood levels of salicylic acid. This effect of DMSO was statistically highly reliable ($F = 76.24$, $df = 1/26$, and $p < 0.001$). More rapid absorption with DMSO is shown by a large difference at the earliest (1.5 hr.) interval. The difference in the shape of the two curves in Fig. 1 resulted in a statistically significant linear function in the interaction between DMSO treatment and time intervals ($F = 12.77$, $df = 1/136$, and $p < 0.001$).

Figures 2 and 3 portray the blood levels over the entire time span of the experiment for the tests with and without DMSO at the two extreme pH levels (2.97 and 10.78). Figure 2 shows the greater effect of DMSO on salicylic acid blood levels at the lowest pH of 2.97 than at the highest pH of 10.78 (Fig. 3) throughout the five time intervals. In spite of the large difference in magnitude of DMSO effect, both figures show that DMSO caused higher blood levels at the early time intervals and a decline in blood levels at the later intervals. This decline was greater and began at an earlier time interval at the highest pH of 10.78. Similarly, the tests with no DMSO showed maximal blood levels at 4.5 hr. at the highest pH of 10.78. The tests with no DMSO showed maximal blood levels at 4.5 hr. at the highest pH of 10.78, whereas the blood levels continued to rise throughout the 7.5 hr. at the lowest pH of 2.97.

To obtain an overall measure of the effect of pH, the blood levels at all five time intervals were averaged together, as shown in Fig. 4. Figures 5 and 6 show the average salicylic acid blood levels as a function of pH at the earliest interval of 1.5 hr. and at the latest interval of 7.5 hr., respectively. The diminished effect of DMSO at the later time interval is evident from a comparison between Figs. 5 and 6. Blood levels of salicylic acid were generally higher at the lowest and the highest pH values than at the intermediate pH levels, both with and without DMSO and at both time intervals.

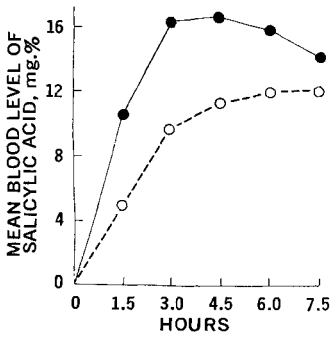

Figure 1—*Mean blood levels of salicylic acid at five time intervals after application of ointment, with all five pH values combined. Key: ●, DMSO added; and ○, no DMSO.*

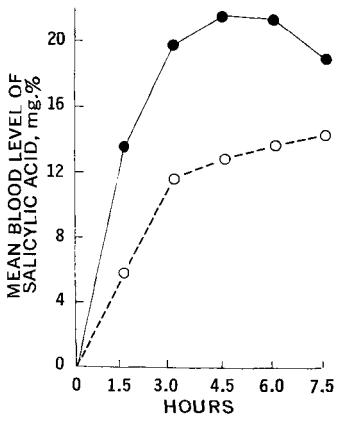

Figure 2—*Mean blood levels of salicylic acid at five time intervals after application of ointment at the lowest pH of 2.97. Key: ●, DMSO added; and ○, no DMSO.*

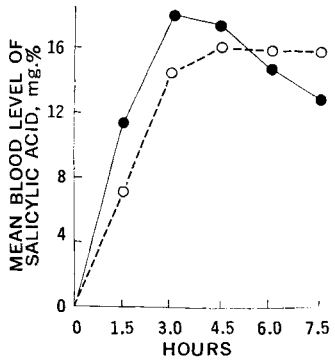

Figure 3—*Mean blood levels of salicylic acid at five time intervals after application of ointment at the highest pH of 10.78. Key:* ●, *DMSO added; and* ○, *no DMSO.*

The decrease in blood levels at the last two time intervals (6.0 and 7.5 hr.) in tests with DMSO treatment (Figs. 1–3) indicates that salicylate available for absorption decreased to the extent that the rate of metabolism and elimination exceeded the rate of absorption at these time intervals. The continuing rise in blood levels at these time intervals after application of the ointment without DMSO further indicates the more gradual rate of absorption in the absence of DMSO. The biasing effect of the decline of blood levels of salicylic acid with DMSO added, at the later time intervals, was removed by using the highest value in the series of five time intervals for each experimental run, as shown in Fig. 7. This analysis resulted in higher average blood levels than in Fig. 4, as would be expected, but otherwise the results in the two figures are rather similar. The lowest blood level occurred at the second lowest pH (4.48), with progressively higher blood levels as the pH increased to 6.80, 9.23, and 10.78. The lowest pH (2.97) also increased blood levels greatly above those obtained with pH 4.48. Statistical analysis of the peak blood levels (Fig. 7) showed a highly reliable effect of DMSO (F = 48.55, df = 1/26, and p <0.001) and a highly reliable quadratic function of pH (F = 10.78, df = 1/9, and p <0.01).

The quadratic function of pH was similar whether or not DMSO was added to the ointment. However, the highest blood levels were obtained at the lowest pH with DMSO and at the highest pH without DMSO. This indicated a tendency for the effect of DMSO to decrease with progressively increasing pH. The test of this differential effect of DMSO, shown in Fig. 7, revealed a linear function for the interaction between DMSO and pH (F = 3.26, df = 1/26, and p <0.10%).

Solubility of salicylic acid was estimated by the amount visible immediately after adding 100 g. of salicylic acid to 1000 ml. of water. Most of the salicylic acid remained in suspension, whereas solution was complete when 150 g. of DMSO was substituted for an equivalent weight of water.

DISCUSSION

The results of this investigation show that at the lowest pH the blood levels of salicylic acid obtained with the emulsion-type ointment base (without DMSO) exceed the blood levels obtainable at the intermediate pH values. As Higuchi (2) theorized, decreasing the pH of a vehicle would be expected to increase the thermodynamic activity of the undissociated form of a weakly acidic drug like salicylate. As Table IV demonstrates, the highest concentration of undissociated salicylic acid is present at the lowest pH. The much lower concentrations of the undissociated species at the three intermediate pH values probably account for the lower levels observed. These findings agree with those of Blank and Gould (5) who noted the increased percutaneous absorption of sodium laurate solutions at pH values at which undissociated lauric acid was present in relatively greater concentration.

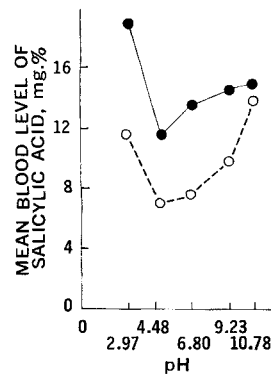

Figure 4—*Mean blood levels of salicylic acid as a function of pH, showing the average for all five time intervals. Key:* ●, *DMSO added; and* ○, *no DMSO.*

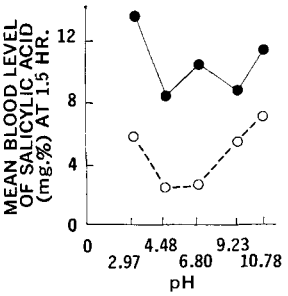

Figure 5—*Mean blood levels of salicylic acid as a function of pH at 1.5 hr. after application of ointment. Key:* ●, *DMSO added; and* ○, *no DMSO.*

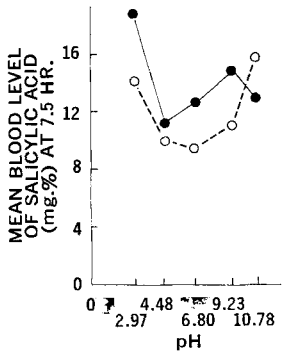

Figure 6—*Mean blood levels of salicylic acid as a function of pH at 7.5 hr. after application of ointment. Key:* ●, *DMSO added; and* ○, *no DMSO.*

Although the three intermediate pH values produced lower blood salicylate levels than the most acidic ointment, a progressive increase in blood levels was observed as the intermediate pH values rose. At the most alkaline pH value, the salicylate levels observed were higher than for any of the other ointments without DMSO, even higher than the levels observed at the most acidic pH. The apparent increase in absorption as the pH values increased might be explained by the more rapid rate of dissolution of salicylic acid that occurs with increasing pH. The rate of dissolution of salicylic acid has been shown to increase by a factor of nearly 15.9 when the pH of an aqueous buffer solution was increased from 1.5 to 6.8, and by a factor of nearly 2 when the pH was increased from 6.8 to 9.0 (33). It is well established that dissolution *in vivo* is often a rate-limiting step in the gastrointestinal absorption process; higher blood levels due to increased absorption can result from a more rapid rate of dissolution of the drug. Although a far greater proportion of salicylate is present in the dissociated form at the more alkaline pH levels, the undissociated species is probably still the absorbing species, because when one molecule of the undissociated species is absorbed (lost from the ointment), another molecule must be formed from the dissociated species to maintain the equilibrium (1).

As Fig. 4 indicates, when the DMSO was incorporated into the ointment bases, the maximum blood levels were achieved at the

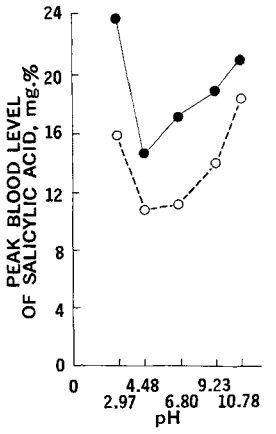

Figure 7—*Mean blood levels of salicylic acid as a function of pH at the time of peak blood level between 1.5 and 7.5 hr. after application of ointment. Key:* ●, *DMSO added; and* ○, *no DMSO.*

Table IV—Comparative Percentages of Undissociated Salicylic Acid in the Aqueous Phase at Each pH and Comparative Total Molar Solubilities of the Drug at Each pH[a]

pH of Aqueous Phase	Percent of Salicylic Acid in Undissociated Form in Aqueous Phase at 25°	Total Molar Solubility at 25°
2.97	50	3.20×10^{-2}
4.48	2.997	5.30×10^{-1}
6.80	0.014	—[c]
9.23	—[b]	—[c]
10.78	—[b]	—[c]

[a] Percentages were determined from the Henderson-Hasselbalch buffer equation (without correcting the activity coefficients). Total solubilities were determined from the equation:

$$pH = pKa + \log \frac{S - S_o}{S_o}$$

where Ka is the dissociation constant of salicylic acid, S is the total solubility (the sum of the molar concentration of salicylic acid in solution plus the molar concentration of salicylate ion), and S_0 is the molar concentration of salicylic acid in water. [b] Essentially complete dissociation. [c] Complete solubility.

lowest rather than highest pH. Therefore, DMSO appears to function as a penetration facilitator of acidic drug molecules more efficiently at the most acidic pH. DMSO completely solubilized the salicylic acid in the aqueous phase, and this increased activity of salicylic acid appears to have been an important factor in the increased percutaneous absorption of the drug. It is not surprising that DMSO enhanced overall percutaneous salicylate absorption to the greatest extent at the most acidic pH, since this is the pH at which undissociated salicylic acid is present to the greatest extent. Thus, solubilization of the less water-soluble (but more absorbable) form into the aqueous phase of the base, from which percutaneous absorption occurs more readily, could have maximal effect.

At the more alkaline pH levels, as dissolution factors become less rate limiting and as a decreasing proportion of undissociated salicylic acid is present, the degree to which the solubilizing effect of DMSO could promote absorption would be expected to diminish. The present results confirm this expectation, but DMSO enhanced absorption even at the most alkaline pH value where its solubilization effect would be minimal. This suggests that DMSO also enhances percutaneous absorption by other mechanisms, possibly by direct action on the skin and cutaneous tissue.

With no DMSO present in the ointment and with all pH values combined, as in Fig. 1, the blood levels gradually rose over the time period from 1.5 to 7.5 hr., reaching an apparent plateau at about 5.5 hr., at which time the rate of absorption apparently approximated the rate of disappearance of drug from the blood. When DMSO was incorporated into the ointments and with all pH values combined, the blood level at 1.5 hr. was more than double that obtained without DMSO. At 3.0 hr., the blood level rose to the extent that it was still almost double the value for no DMSO. The steeper initial slope observed with DMSO indicates an increased absorption rate following initial application of the drug. The subsequent decline might indicate that with DMSO the store of absorbable salicylic acid is exhausted more rapidly. Another possible reason for the decline is that the DMSO, which is itself absorbed rapidly, is no longer available to facilitate salicylate absorption. Thus, it is probable that the effect of DMSO is underestimated at later time periods, when its rapid absorption has exhausted the concentration of the solvent.

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