Ability of Certain Additives to Influence the Absorption of Salicylic Acid from Solutions in an *In Vivo* Study: Preliminary Report

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The influence of polysorbates, sucrose, polyethylene glycols (PEG), propylene glycol, and dimethylsulfoxide (DMSO) on the absorption of salicylic acid has been studied. An immersion technique utilizing the frog was used. Results were analyzed by the Student *t* test. Certain concentrations of additives were seen to enhance absorption.

In recent years interest has been growing in the area of pharmaceutical research involving factors that influence drug absorption. Certain theories or concepts have been advanced concerning drug absorption. Shore *et al.* (1) reported certain drugs cross the intestinal epithelium chiefly in the unionized form and these workers advanced the pH-partition theory to explain the passive absorption of drugs. Various investigators (2–5) have found that oil-soluble drugs penetrate the skin more readily than water-soluble substances.

It was found that the frog, *Rana pipiens*, absorbed salicylic acid from solutions (6). The object of this study was to determine the effect of certain additives on the absorption of salicylic acid utilizing this immersion technique.

EXPERIMENTAL

Ten frogs were used in each determination. Each frog was placed in 500 ml. of the drug-additive solution and at 20-min. intervals the solutions were assayed for salicylic acid content using a Beckman DU spectrophotometer at a wavelength of 297 mμ. Samples were quickly returned to the solutions in order to maintain volume. It was determined that none of the additives interfered with the assay procedure. The solutions were 2.5×10^{-4} *M* with respect to salicylic acid.

It has been pointed out (6) that the absorption of salicylic acid by the frog in the manner described appears to conform to the pH-partition theory of passive absorption of drug in that the molecular form of drug is favored over the ionic. Proper maintenance of pH during such a study is therefore recognized to be of importance. It was found that most of the solutions except those containing the polysorbates exhibited a slight increase in pH (averaging about 0.15 pH units) during the second hour of the measurements. Part of this increase can be accounted for by the reduction in salicylic acid concentration as the drug is absorbed. Several buffer systems were tried. All were rejected because they either were deleterious to the frogs, or they greatly changed the rate of absorption possibly indicating an effect on the permeability of the skin of the frog. It appeared that the slight increase in pH observed did not appreciably influence the results of the study.

The aqueous solubility of salicylic acid in the presence of the additives was determined in an effort to correlate this property to the rates of absorption.

RESULTS AND DISCUSSION

Additives studied included polysorbate 80 USP, dimethylsulfoxide, sucrose, propylene glycol USP, polyethylene glycol 300 NF, and polyethylene glycol 4000 USP. The concentration of each additive was varied to observe this influence.

Figure 1 shows the effect of various concentrations of polysorbate 80 on the absorption of salicylic acid from solutions. This surfactant is seen to alter the rate of absorption of salicylic acid. The 0.1% concentration of additive has the greater effect in increasing absorption whereas the 1.0% polysorbate 80 is seen to have little effect for the first hour and then to inhibit absorption thereafter. Statistical analyses of the results by the Student *t* test at the 2-hr. mark show significant differences between control and the solutions containing 0.1% polysorbate 80 but not with the 0.5% additive solution. Levy *et al.* (7) found low concentrations of polysorbate 80 increased the absorption rate of certain barbiturates by goldfish, but higher concentrations of the surfactant inhibited absorption. Table I shows the aqueous solubility of salicylic acid to be greater in the presence of polysorbate 80. Each increase in surfactant concentration produced an increase in solubility. The surface tension lowering properties of polysorbate 80 prob-

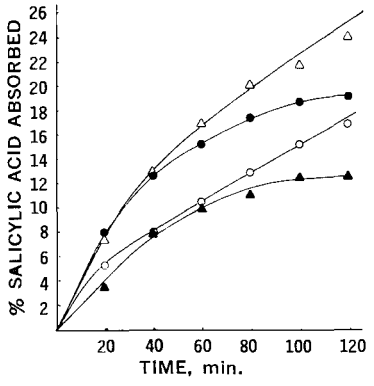

Fig. 1—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5×10^{-4} M and salicylic acid, 2.5×10^{-4} M in the presence of: △, 0.1% polysorbate 80; ●, 0.5% polysorbate 80; ▲, 1.0% polysorbate 80.

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TABLE I—SOLUBILITY OF SALICYLIC ACID AT 27° IN THE PRESENCE OF ADDITIVES

Solvent, %	Solubility, Gm./100 ml.
Water	0.275
Polysorbate 80, 0.1	0.275
Polysorbate 80, 0.5	0.367
Polysorbate 80, 1.0	0.458
DMSO, 0.5	0.275
DMSO, 1.0	0.275
DMSO, 5.0	0.321
Sucrose, 0.5	0.275
Sucrose, 1.0	0.275
Sucrose, 5.0	0.275
Propylene glycol, 0.5	0.275
Propylene glycol, 1.0	0.275
Propylene glycol, 5.0	0.275
PEG 300, 0.5	0.275
PEG 300, 1.0	0.275
PEG 300, 5.0	0.343
PEG 4000, 0.5	0.275
PEG 4000, 1.0	0.275
PEG 4000, 5.0	0.390

ably permit better wetting action by the solution, thereby enhancing absorption of the salicylic acid.

Dimethylsulfoxide has received wide acclaim recently for its alleged ability to penetrate the skin. The use of this solvent as a vehicle for enhancing absorption of drugs remains a possibility. DMSO was found in this study to be more effective than any other additive observed in increasing the rate of absorption of salicylic acid by the live frog. All concentrations of DMSO significantly increased the amount of salicylic acid absorbed at the end of 2 hr. (Fig. 2). There appears to be an optimum concentration of DMSO, however, which will increase absorption just as was true with the polysorbate 80. Morain and his co-workers (8) observed DMSO to increase the permeability of frog skin to mannitol, urea, and other substances. The solubility of salicylic acid is seen to be increased by DMSO only by the 5.0% concentration.

The presence of sucrose (Fig. 3) increases the rate of absorption of salicylic acid by the frog but does not influence the solubility. All differences between control and solutions containing additives were significant at the 2-hr. limit. Again there appears to be an optimum concentration of additive for increasing absorption with 1% sucrose having the greatest effect.

As seen in Fig. 4 propylene glycol significantly increases the absorption of salicylic acid. As the concentration of additive is increased, however, the effect is less pronounced. No change in solubility was noted.

Figure 5 shows the 1.0% concentration of polyethylene glycol 300 to be more effective in increasing absorption than the 0.5% (not significant) or the

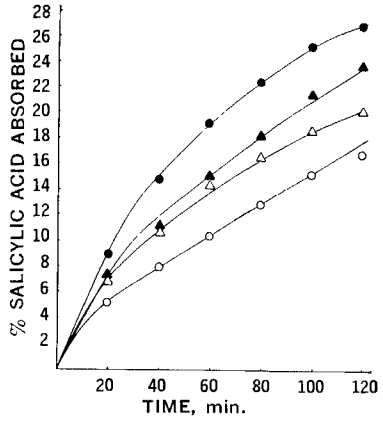

Fig. 3—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5 × 10⁻⁴ M and salicylic acid, 2.5 × 10⁻⁴ M in the presence of: △, 0.5% sucrose; ●, 1.0% sucrose; ▲, 5.0% sucrose.

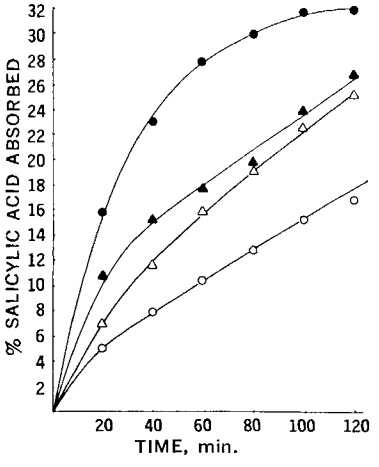

Fig. 2—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5 × 10⁻⁴ M and salicylic acid, 2.5 × 10⁻⁴ M in presence of: △, 0.5% DMSO; ●, 1.0% DMSO; ▲, 5.0% DMSO.

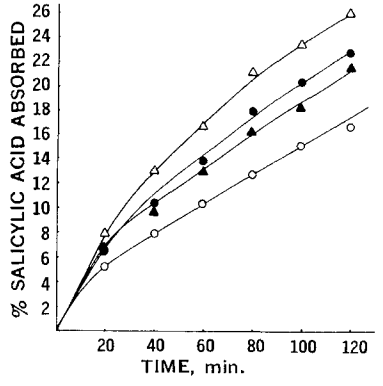

Fig. 4—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5 × 10⁻⁴ M and salicylic acid, 2.5 × 10⁻⁴ M in presence of: △, 0.5% propylene glycol; ●, 1.0% propylene glycol; ▲, 5.0% propylene glycol.

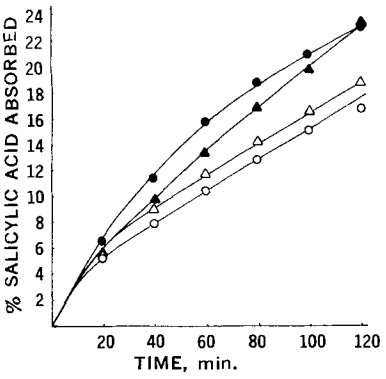

Fig. 5—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5 × 10^{-4} M and salicylic acid, 2.5 × 10^{-4} M in presence of: △, 0.5% polyethylene glycol 300; ●, 1.0% polyethylene glycol 300; ▲, 5.0% polyethylene glycol 300.

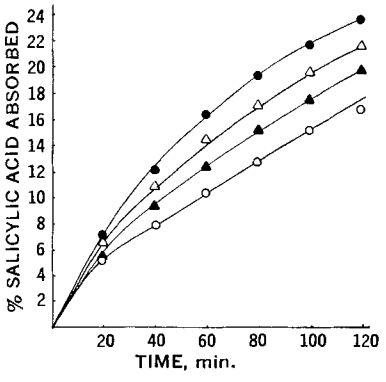

Fig. 6—Percent of salicylic acid absorbed over 2-hr. period when ten frogs each were placed in 500 ml. of aqueous drug solution. Key: ○, salicylic acid, 2.5 × 10^{-4} M and salicylic acid, 2.5 × 10^{-4} M in presence of: △, 0.5% polyethylene glycol 4000; ●, 1.0% polyethylene glycol 4000; ▲, 5.0% polyethylene glycol 4000.

5.0%. Only the 5.0% concentration of the additive influenced solubility which was slightly increased.

Polyethylene glycol 4000 significantly increased the absorption of salicylic acid (Fig. 6) with the 1.0% concentration again being the most effective. The solubility was increased only by the 5.0% additive solution.

Efforts at linearizing all the curves in the same manner were not successful. It was previously shown (6) that the absorption rate for salicylic acid from solutions using this technique appeared to be first order. This did not prove to be true, however, for most of the solutions containing the additives especially in the instances in which the absorption rate was greatly increased. Throughout the experiments the concentration of drug in the immersion media greatly exceeded the concentration of drug in the frogs. Considering this and the fact that a large volume of drug solution was used indicates that a fairly constant concentration gradient of drug was maintained after the initial 20-min. period of absorption. This explains the apparent zero-order absorption process seen for some of the solutions after the first 20 min. Several of the plots, namely for propylene glycol and polyethylene glycol 4000 additives, show similar slopes for all concentrations of additive indicating similar absorption rates after the initial absorption period.

The failure of certain of the data to show zero-order or first-order characteristics might be due to excretion of the drug by the frog toward the end of the experiments. This explanation is perhaps plausible for those additives that showed the greatest ability to enhance absorption after which the drug may have been partially excreted.

Current studies are being conducted to determine what part complexation, pH, concentration of drug, and other parameters are playing in the absorption of salicylic acid by the frog.

SUMMARY AND CONCLUSIONS

1. The effect of six additives at various concentrations on the absorption of salicylic acid from solutions has been studied using the frog as the test animal. The solubility of salicylic acid in each solvent was determined.

2. The additives were seen to enhance absorption of salicylic acid significantly in most cases, however, there appeared to be an optimum concentration for most of the additives.

3. The influence of various adjuvants on the absorption of the active ingredient of a pharmaceutical formulation should be carefully considered.

4. The frog may be useful for studying drug absorption.

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