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# PERCUTANEOUS ABSORPTION OF SALICYLIC ACID IN RABBITS

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The pharmacokinetic parameters defining the percutaneous absorption of salicylic acid in rabbits have been investigated. A one compartment open model with apparent first order absorption was found to describe adequately the blood level data.

## INTRODUCTION

Topical application of salicylic acid preparations is a well established practice in dermatology, particularly for the long term treatment of psoriasis. Although the in-vitro release rate and the extent of percutaneous salicylic acid absorption in-vivo from various ointment bases has been thoroughly studied,[1-3] there is, as Taylor and Halprin[4] and Cooper et al.[5] have pointed out, a paucity of pharmacokinetic data characterizing the absorption of percutaneously applied salicylic acid. This paper reports estimates of the pharmacokinetic functions which define the topical absorption of salicylic acid in rabbits.

## EXPERIMENTAL

Materials - Hydrophylic ointment U.S.P. (Ruger Chemical Co., Irvington, NJ), obtained commercially, was milled (Hammonia Ointment Mill, Josef Deckelmann, Aschaffenburg, W. Germany) to improve consistency and the ease of application. To provide a uniform particle size, salicylic acid (Fisher Scientific Co., Fairlawn, NJ), and urea (Fisher Scientific Co., Fairlawn, NJ), were passed through an 80 mesh sieve prior to use. All other chemicals were analytical reagent grade, and were used as received.

Salicylic acid in hydrophilic ointment (10% w/w) (I) and salicylic acid (10% w/w) and urea (10% w/w) in hydrophilic ointment (II) were prepared the day of application.

Tungstic acid reagent was prepared by mixing 10 ml. of 10% aqueous sodium tungstate solution and 80 ml. of 1/12 N sulfuric acid. The reagent was prepared fresh the day samples were analyzed.

Methods - Female New Zealand rabbits, age approximately five months and weighing between two and three kilograms, were housed in humidity and temperature controlled quarters and maintained on a diet of rabbit chow and water ad lib. Rabbits were placed in restraining cages for successively longer periods of time over a span of several days for conditioning before being used for an experiment.

The hair on the ventral side of the animal from the forelegs to the hindlegs was carefully removed with animal clippers two to three hours prior to application of a test vehicle, taking care not to cut or otherwise visibly damage the skin surface.

An intermittent infusion set, 21 gauge, with a 15.9 mm (5/8") needle (Butterfly-21, Intermittent Infusion Set, Abbott Laboratories, Chicago, IL) was inserted into the central ear artery, immediately prior to application of the test vehicle. The infusion line was filled with a dilute, 100 units per ml., heparin solution whenever samples were not being collected. This technique allows for repetitive sample collection without the need for numerous venipunctures. A blood sample was collected before application of the ointment to serve as a pretreatment control.

Ten grams of ointment was spread over a standard, rectangular, 7 cm. x 13 cm. (1.75 x 5.11") template following the technique of Stollar et al.[1] The template was applied to the shaved area of the animals abdomen and held in place with adhesive tape. The rabbit was then placed in a restraining cage for the duration of the experiment. Water was provided ad lib., food was given or withheld as reported in each study. Two ml. blood samples were taken, the serum separated and refrigerated at 4°C until assayed. Blood samples were taken immediately prior to application of a test vehicle, during the eight hour application period, and for eight hours following the removal of the vehicle template.

Total salicylic acid was assayed by the spectrofluorometric method of Saltzman.[6] Nine and one-half ml. of tungstic acid reagent was added to 0.5 ml. of plasma, this solution was shaken and the reaction allowed to develop for at least ten minutes. The mixture was then filtered and 5 ml. of filtrate was added to 7 ml. of 10 N NaOH. The fluorescence of the resulting solutions was determined within thirty

minutes using a fluorometer (Turner Model 111, G. K. Turner Associates, Palo Alto, CA) fitted with a 405 nm primary filter and a secondary, sharp cut filter passing all light greater than 455 nm. Salicylic acid concentrations were determined by comparison to a standard curve. Plots of fluorescence as a function of standard salicylic acid concentrations were linear over the range 0.5 to 20 mg.% (correlation coefficient 0.99, n = 8).

## RESULTS AND DISCUSSION

Salicylic acid plasma levels as a function of time for a fasted animal treated with (I) for eight hours is shown in Figure 1. Peak plasma salicylic acid concentrations observed were between 10 mg.% and 18 mg.%, and were attained within four to six hours of ointment application. It appeared that in most cases the absorption process was essentially completed prior to ointment removal. Plasma salicylic acid concentrations were detectable (greater than 0.5 mg.%) for at least twelve hours after the removal of the ointment.

The plasma salicylic acid concentrations in the post-absorptive phase appears to decline monoexponentially (Figure 1). The elimination rate constant, $k_e$, was calculated from a log-linear regression of the plasma salicylic acid concentration as a function of time. The elimination rate constant for eight rabbits treated with (I) for eight hours was determined to be $0.41\ hr^{-1} \pm 0.27\ hr^{-1}$ (s.d.) (Table I).

The Wagner-Nelson method is commonly used to characterize the absorption process.[7] This technique is based on the one-compartment open model, and can be used to obtain data from which the order of

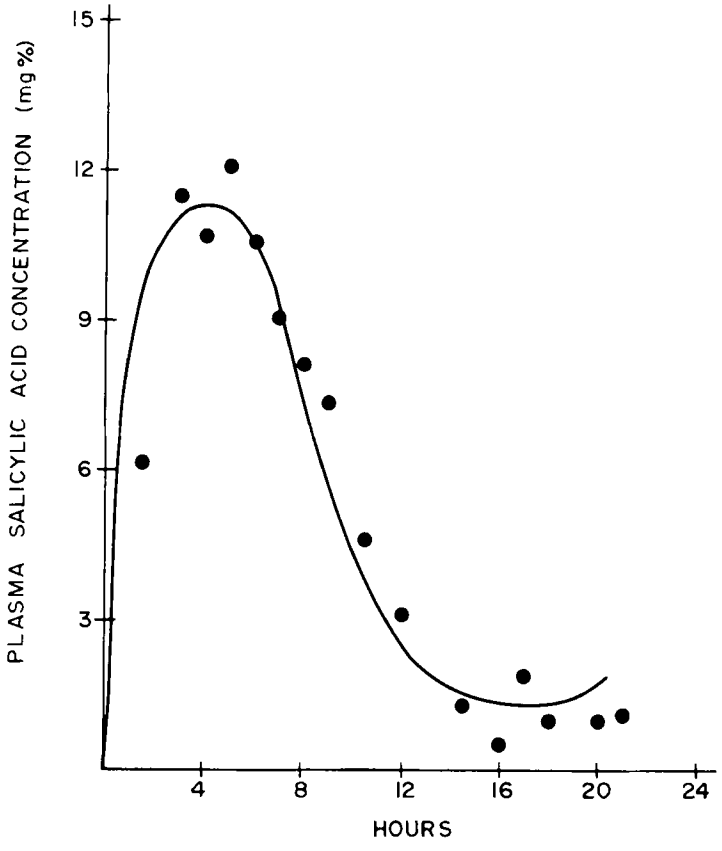


Figure 1

Plasma salicylic acid concentration as a function of time. Eight hour application of ointment (I).

absorption and the appropriate rate constant can be calculated. The Wagner-Nelson equation is

$$At/V = Ct + k_e \int_0^t Cpdt \qquad \text{(Eq. 1.)}$$

Where At/V is the amount of drug in the body at time t, divided by the volume of distribution V, Ct

TABLE I Pharmacokinetic parameters derived from the topical absorption of Salicylic Acid in the Rabbit

| TREATMENT | |
|---|---|
| Ka | ($hr^{-1}$ ± s.d.) |
| Wagner-Nelson | 0.34 ± 0.28 |
| NONLIN | 0.25 ± 0.07 |
| Ke | ($hr^{-1}$ ± s.d.) |
| Elimination Plot | 0.41 ± 0.27 |
| NONLIN | 0.29 ± 0.07 |
| Lag Time | (hr ± s.d.) |
| Hours | 1.09 ± 0.16 |

represents the concentration of drug in the blood at time t, and the integral $k_e \int_0^t Cpdt$ represents the cumulative amount of drug eliminated.

If the plot of the logarithm of the change in At/V versus time is linear, the absorption process can be characterized as first order and an absorption rate constant determined. A representative plot is shown in Figure 2. Such plots were linear in all cases

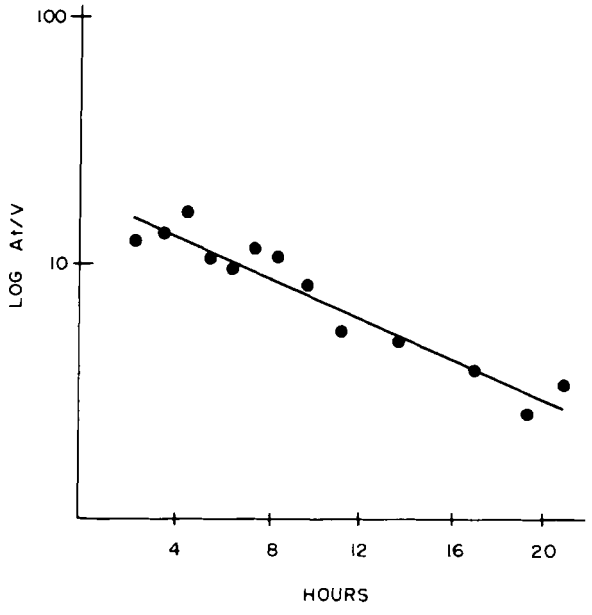


Figure 2
Absorption rate of salicylic acid calculated by the Wagner-Nelson method.

characterizing the absorption of topically applied salicylic acid as a first order process. An average absorption rate constant of 0.34 $hr^{-1}$±0.28 $hr^{-1}$ (s.d.) was obtained (Table I).

Since the absorption rate was characterized as first order, fitting the one compartment open model, the data was treated by a nonlinear least squares regression analysis program (NONLIN)[8] to refine further the estimates of $k_a$ and $k_e$. An appropriate set of equations for the one compartment open model with first order absorption was written for the IBM 370/155 digital computer. The parameters obtained graphically were used as initial estimates. Conver-

gence of parameters to a best fit occurred within 20 iterations for all data sets, and the regression coefficients were greater than 0.9. The values of $k_a$ and $k_e$ calculated directly by graphic methods previously described and by NONLIN (Table I) were not found to be statistically different (Student's t test, $p = 0.05$), most likely due to the large standard deviations in absorption and elimination rates.

A lag time, defined as the time required for the absorption process to become pseudo first order, was needed to describe the percutaneous absorption of salicylic acid. By expressing the amount of drug absorbed at time t as a percentage of the total amount absorbed (A/V) and subtracting from 100 the percent remaining to be absorbed can be calculated. A plot of the percent remaining to be absorbed as a function of time was linear with a slope equal to $k_a$ (Figure 3). Performing linear regression analysis and solving for the time at which 100% of drug remains to be absorbed yields the lag time. A value of 1.09 hr ± 0.16 hr (s.d.) was obtained (Table I).

In contrast to previous studies in which animals were fasted throughout an experiment, two animals were fed approximately 30 grams of rabbit chow fifteen hours following the application of ointment (I). A plot of plasma salicylic acid concentration as a function of time for an animal fed is shown in Figure 4. Absorption and elimination rate constants calculated from the plasma salicylic acid concentrations through the first sixteen hours are not significantly different from those calculated using fasted animals. However, whereas in fasted animals plasma salicylic acid levels remained low, less than 2 mg%, for the remainder of the collection period (Figure 1), a

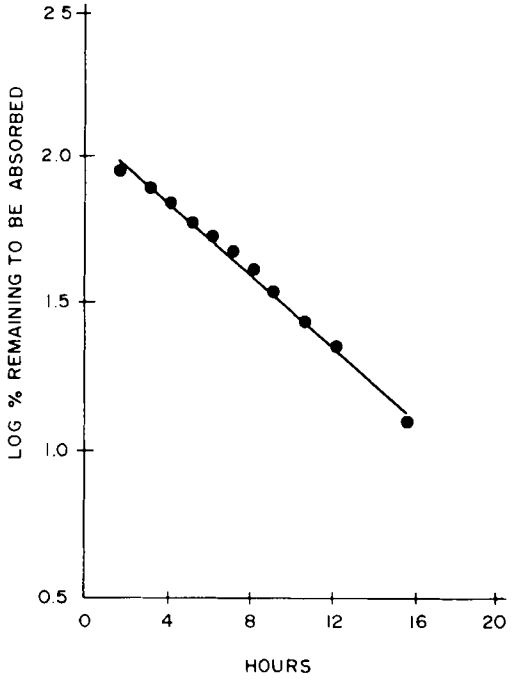


Figure 3
Percent salicylic acid remaining to be absorbed.

second peak was observed in both the animals fed during the collection period. In both cases the plasma concentrations prior to feeding were consistent with those observed in fasted animals. Secondary peak concentrations above 6.5 mg% were detected 3 to 5 hours following the feeding. These peaks correspond to times approximately nine and twelve hours after the removal of the ointment. As a result, it is unlikely that the second peak can be attributed to additional absorption of salicylic acid through the skin. This peak seen only in animals fed during an experiment may perhaps be attributable to biliary recycling.

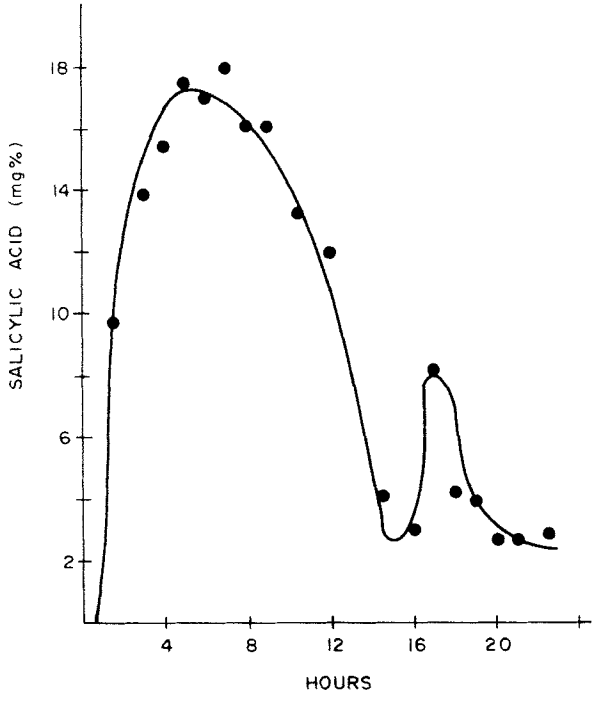


Figure 4
The effect of feeding on the plasma salicylic acid concentration time profile following the topical administration of salicylic acid.

Recycling of drugs excreted into the bile in unchanged form and for metabolites is possible, if they are either absorbed or modified by the gut flora, particularly after splitting of conjugates.[9] The form of drug which is excreted into the bile also affects recycling, drug which form glucuronides have been shown to undergo recycling, since the glucuronide can be readily cleaved to form the free drug in the bile. Salicylic acid in man is metabolized by the liver, and its metabolites, salicylacylglucuronide,

salicylphenolicglucuronide, salicyluric acid, and gentisic acid are found in the bile.[10] If the unchanged drug and the glucuronide account for the greater portion of the drug in the bile of rabbits as in man, it is quite likely that biliary recycling occurs.

The results of biliary recycling of drugs is commonly seen as either a series of irregularly spaced peaks in the declining portion of a blood concentration as a function of time plot or simply as a prolonged elimination phase.[9] Since the rabbit, unlike some species, is known to store bile and release it upon stimulation such as food,[11] it is possible that the stimulus provided by the small amount of food caused the release of bile which in turn led to the reabsorption of salicylic acid.

Studies designed to investigate the possible utility of urea to enhance percutaneous absorption did not reveal any effect on the rate of absorption of topically applied salicylic acid.

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