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Recent advances in percutaneous absorption using the rhesus monkey model

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Synopsis

PERCUTANEOUS ABSORPTION in non-primate laboratory animals has been shown to be different from man. This report REVIEWS STUDIES in which skin absorption in the RHESUS MONKEY was shown to be similar to man. This animal MODEL was then used in a series of studies on factors affecting skin absorption.

The amount of compound absorbed through the skin was shown to be dependent on the topical concentration applied to the skin. Also, the frequency of daily application affected absorption; a single large dose had greater absorption than equivalent divided doses applied at more frequent intervals. Additionally, acute versus chronic topical dosing was compared. Penetration is routinely measured by acute administration. However, the absorption of hydrocortisone significantly increased during chronic administration. This suggests that chronic application of hydrocortisone alters the penetration barrier resulting in enhanced absorption.

Salicylic acid was thought to enhance the penetration of other compounds through its keratolytic action. However, in a single dose application study, the addition of salicylic acid, at equal to or ten times the concentration of hydrocortisone, did not enhance the absorption of hydrocortisone.

Toxicity from topical compounds is critical in the newborn. The same high percentage of a steroid that can be absorbed through the skin of an adult can be absorbed in a newborn. This could become critical in a newborn because the ratio of surface to body weight is three times that of the adult. The therapeutic ratio probably is lower in the newborn than in the adult when the compound is applied topically.

INTRODUCTION

This paper discusses some recent work using the rhesus monkey as an animal model for percutaneous absorption. First we compare percutaneous absorption in the rhesus monkey and other animal models to that in man. We then review recent studies performed with the rhesus in which the experimental design was changed from the usual procedure of applying a single topical dose, to designs which may be more

relevant to the clinical situation. This will include changes in topical concentration, a single daily topical dose versus daily divided doses, and the effect of chronic application of a topical compound. The final part of this paper will review a study involving topical application of a mixture (hydrocortisone and salicylic acid) and a study involving skin absorption in the newborn.

METHODOLOGY

Absorption was quantified on the basis of the percentage of radioactivity excreted in urine following application of a known amount of the labeled compound to the skin (1). Female rhesus monkeys (4 to 8 kg), trained for metabolic studies, were randomly selected from the colony. The only criteria for selection was that the animal was healthy and clear of any previously administered ^{14}C-compound. Compound labeled with radioactive carbon (^{14}C) was applied to lightly clippered shaved skin. Previous experience with testosterone absorption showed that this light clipper shaving did not alter penetration in this animal (1).

For topical application each dose contained approximately 5 μCi of radioactivity. The area was not occluded and the application area was constant each study. Each monkey was placed in a metabolism chair for the first 24 hr of the study. The hands of the monkey were secured to the sides of the chair to avoid its wiping off the applied compound. The animals were not anesthetized during the study. Compound was applied to the skin in organic solvent or cream vehicle. Where an organic solvent was used, the solvent was quickly evaporated by gentle blowing. For the next 24 hr urine was collected in a container below the metabolism chair. Then the site of application was washed with soap and water. After the topical application period each monkey was returned to a metabolism cage to continue the urine collection. In the metabolism chair the site of application was totally separated with a barrier from the area of urine collection. This insured against any possibility of chemical falling off the skin and contaminating the urine.

For each compound studied, an intravenous dose of the labeled compound was administered and urine collected. The percentage of dose excreted following intravenous administration was used to correct for excretion of radioactivity by other routes (feces, CO_2) and for compound retained in the body (1).

The above is a generalized summary of the procedures used. Individual studies contained some additional methods or adaptions to the general procedure. These changes are contained in the individual papers which are referenced.

RESULTS AND DISCUSSION

PERCUTANEOUS ABSORPTION IN THE RHESUS MONKEY COMPARED TO MAN

The use of animal species which pharmacokinetically resemble man is most desirable in metabolic studies. In a previous comparative study Bartek, La Budde and Maibach (2) showed that the absorption of compounds differed between man and other species, with (for the few compounds examined) pig skin having the closest absorption to that of human skin. In our first comparative study between the rhesus monkey and man,

percutaneous absorption of hydrocortisone, testosterone and benzoic acid showed the same absorption in both species (1). This suggested that the rhesus monkey may be a suitable animal model.

In this study (1) we used a single topical concentration (4 $\mu g/cm^2$) in the rhesus monkey to compare with the published human data using the same concentration (3,4). As part of a study on the relationship of topical dose and percutaneous absorption, we were fortunate to have data to compare between the rhesus monkey and man (5). Figure 1 shows the absorption of hydrocortisone, testosterone and benzoic acid after

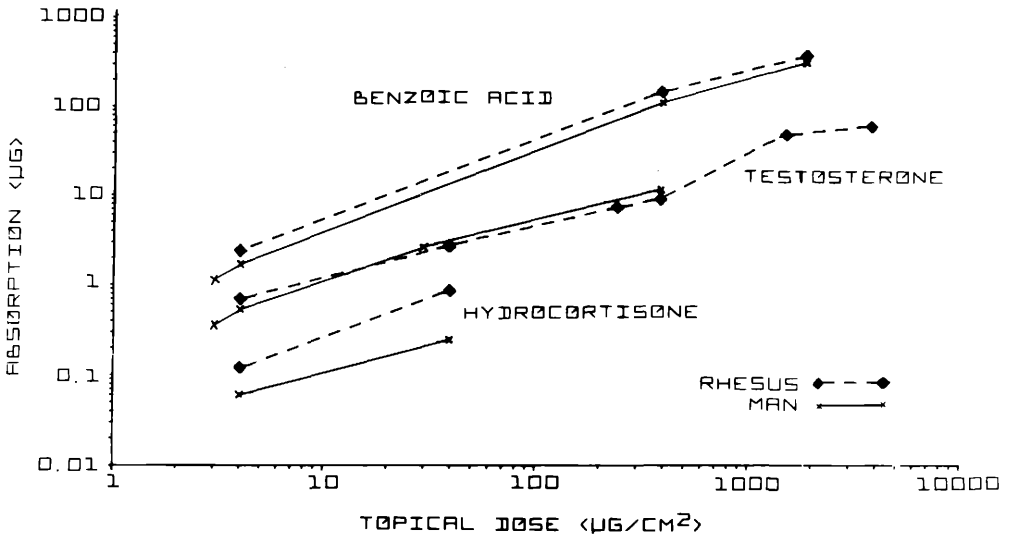

Figure 1. Comparison between rhesus monkey and man of compounds absorbed (μg) after topical application of increasing doses ($\mu g/cm^2$) of testosterone, hydrocortisone and benzoic acid. Reproduced from Wester and Maibach (5) with permission of the Williams & Wilkins Co.

topical application of increasing doses. In both species the high penetrant (benzoic acid) is distinguishable from the medium penetrant (testosterone), which in turn is distinguishable from the low penetrant (hydrocortisone). Both the rhesus monkey and man show an increase in the mass of material absorbed (μg) as the topical dose ($\mu g/cm^2$) was increased. This was true for each of the three compounds tested. With equivalent doses, the absorption was similar between the rhesus and man.

In the previous studies (1,5) the anatomic site of topical application for both the rhesus monkey and man was the ventral forearm. However, in the clinical situation a variety of anatomic sites are utilized. We know that in man percutaneous absorption varies with the site of application (6,7). We therefore studied regional variation in the rhesus monkey, and with testosterone ranked the sites from most absorption to least absorption as vagina >> scalp > cheek = ventral forearm > chest (8). The ratio of absorption for scalp to forearm was 2.3. In man the ratio of absorption for scalp to forearm was 3.7 for parathion (7) and 3.5 for hydrocortisone (6). There were no human data with which to compare the other sites studied in the rhesus monkey.

The in vivo data suggest that the rhesus monkey may be a suitable model for

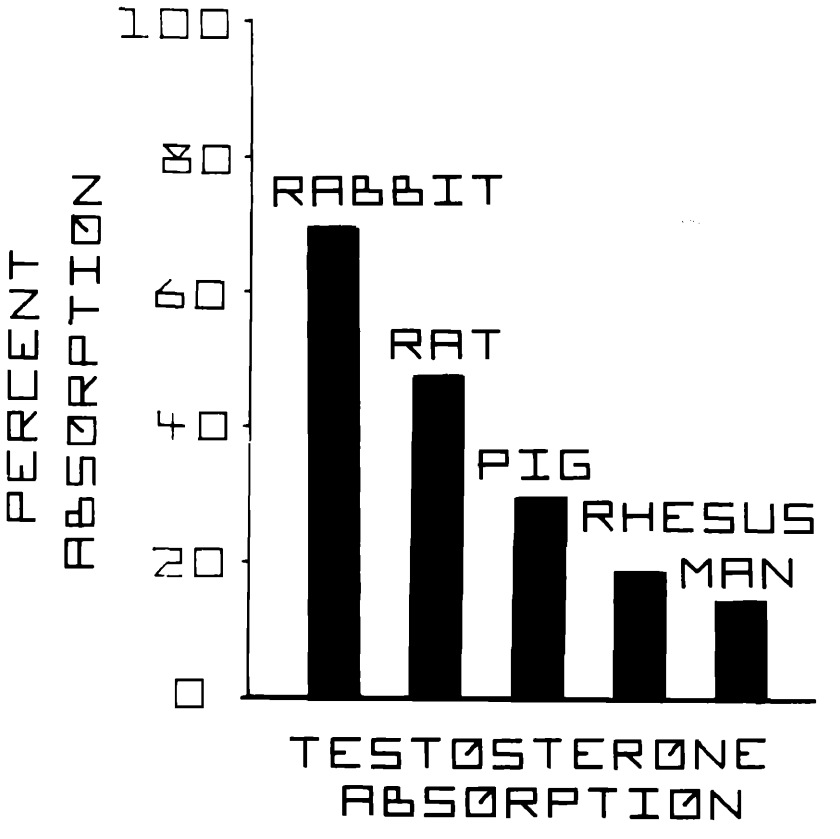

Figure 2. Percutaneous absorption of testosterone (4 $\mu g/cm^2$) by rabbit, rat, miniature swine, rhesus monkey and man. Reproduced from Wester and Maibach (10) with permission of Academic Press, Inc.

percutaneous absorption with some relevance to man (9,10). Small laboratory animals have high percutaneous absorption values when compared to the pig, rhesus monkey and man (Figure 2). Other non-human primates have been compared to man. Bartek and LaBudde found generally a good comparison between the squirrel monkey and man (11). Sinha, Shaw and Weber (12) found the percutaneous absorption of diflorasone diacetate to be greater in the Cynomolgus monkey than in man. However, they used different anatomic sites of application for the two species. Ranking of skin permeability of different species as determined in vitro show monkey, pig and chimpanzee skin to be closer to human skin than skin of many other species (13). Generally, the ranking of skin permeability by specie in vitro was in close agreement to the ranking of skin permeability by specie in vivo (10).

FACTORS AFFECTING PERCUTANEOUS ABSORPTION AS STUDIED USING THE RHESUS MONKEY MODEL

Percutaneous absorption studies are usually conducted using a single application. By some analytical means the amount absorbed is determined and the percentage of absorption of that compound is calculated. This is fine for the limits of the study, but the question remains as to its relevance to the clinical situation. A topically

administered compound (prescribed or exposed contamination) can consist of various dose levels, can be applied more frequently than once per day, and the topical exposure can be on a chronic basis. Some of our studies relating to these conditions follow.

The effect of changes in topical concentrations was studied in both the rhesus monkey and man (5). Compounds compared were testosterone, hydrocortisone and benzoic acid, at up to 1000-fold changes in concentration. The results show a relationship between the concentration of the topical dose and skin absorption. The efficiency of absorption (percent) as well as the mass of material absorbed (micrograms) changed as the topical dose changed (Figure 3). Secondly, as pointed out previously in Figure 2, as the topical concentration was increased, the total amount of compound (micrograms) absorbed always increased. This is consistent with published in vitro data (14). Therefore, it is possible for greater than 100-fold increases in skin penetration to be achieved by increasing the concentration per unit surface area.

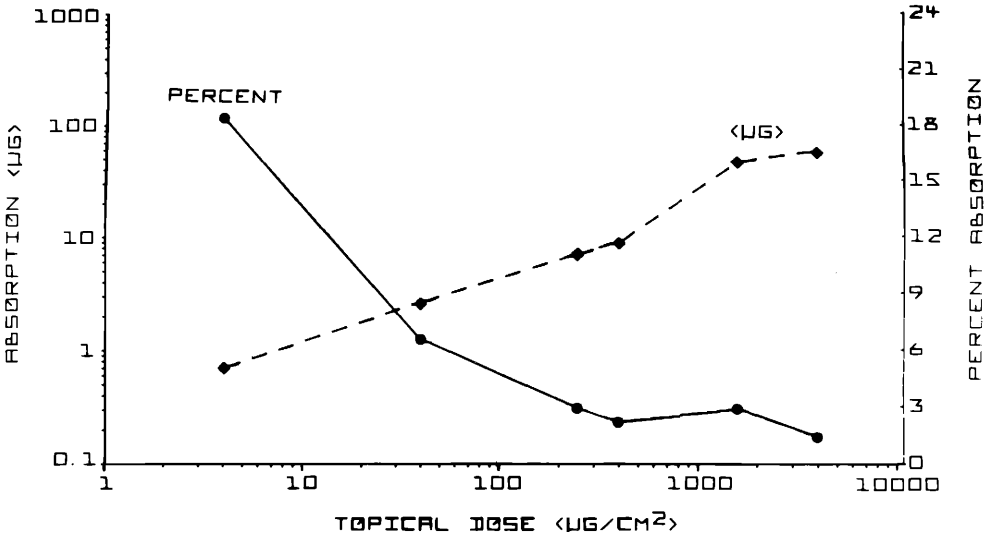

Figure 3. Percutaneous absorption of increasing topical doses of testosterone in the rhesus monkey. Reproduced from Wester and Maibach (5) with the permission of the Williams and Wilkins Co.

Another study determined the percutaneous absorption of compound when applied as a single dose or on a repetitive basis. The study was first done with hydrocortisone (15) and then confirmed using testosterone (8). In both studies there was no substantial difference in total absorption when 13.3 $\mu g/cm^2$ was applied as a single dose or when 13.3 $\mu g/cm^2$ was applied three times, totaling 40 $\mu g/cm^2$. However, when 40 $\mu g/cm^2$ was applied as a single dose, absorption was significantly increased over 13.3 $\mu g/cm^2$ applied either once or three times. The practical ramification is that it is possible that one application of a high concentration may be in fact more effective than several applications of a lower concentration.

In the studies of repetitive dosing (8,15) the site of application was either unwashed or washed with soap and water between successive dose applications. In the testosterone study (8) there was no difference in absorption between the washed and unwashed

skin. However, washing the skin between repetitive hydrocortisone applications statistically produced an increase in penetration (15). In prior studies with pesticides (unpublished observations) this phenomenon had been repeatedly observed. This suggests that when we wash off compounds to decrease the chance of toxicity, we may be doing the exact opposite, that of increasing the risk of toxicity. Perhaps it is that the soap and water wash hydrates the skin and this would account for the increased absorption when the skin is washed between applications.

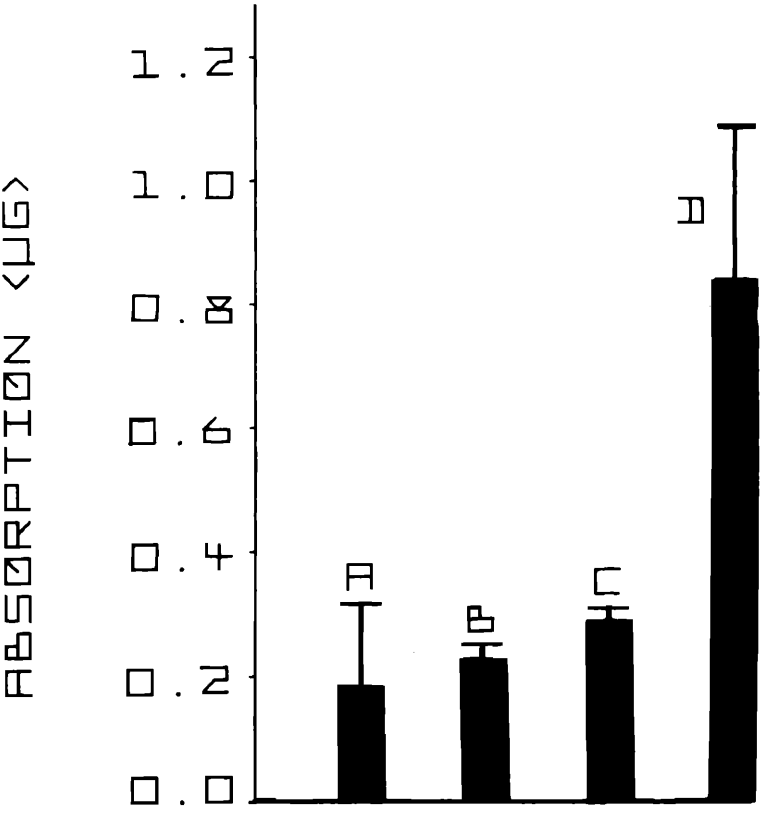

Figure 4. Effect of frequency of application on percutaneous absorption of hydrocortisone from ventral forearm of rhesus monkey. Ordinate is micrograms absorbed. A is 13.3 $\mu g/cm^2$ hydrocortisone applied once; B and C are 13.3 $\mu g/cm^2$ applied three times (total to 40 $\mu g/cm^2$) with area washed between applications for C; D is 40 $\mu g/cm^2$ applied once. Each bar graph is the mean + standard deviations of the mean. Figure is reproduced from the data of Wester, Noonan and Maibach (15).

Percutaneous absorption studies have previously involved acute (single) administration of compound; whereas chronic administration is the relevant clinical situation. A study was done to compare percutaneous absorption of hydrocortisone after acute and chronic administration (16). The experimental design was to first apply [^{14}C]-hydrocortisone, followed by chronic administration of non-radioactive hydrocortisone, and apply [^{14}C]-hydrocortisone when ^{14}C from the first application reached background level. The absorption of hydrocortisone significantly increased during

chronic administration, whether applied in acetone vehicle or as a 0.9% Eucerin® cream. Percutaneous absorption (acetone vehicle) on day 8 of application was increased 144% over that on day 1 application. The greatest increase in urinary excretion was at 24 hr. Applying only the acetone vehicle for seven days (control study) did not significantly increase (5%) hydrocortisone absorption. In a cream vehicle, absorption on day 8 was increased 275% with chronic hydrocortisone application. Again, the greatest increase in urinary excretion was at 24 hr. These results suggest that chronic application of hydrocortisone alters the penetration barrier resulting in enhanced penetration.

SALYCYLIC ACID AND HYDROCORTISONE

Dermatologists have long held that salicylic acid enhances the efficacy of other pharmacological agents, one of which is hydrocortisone. It has been assumed that the keratolytic action of salicylic acid enhances the penetration of other agents. An in vitro study by Polano and Ponec (17) did indeed show this.

To determine the effect of salicylic acid on hydrocortisone penetration in vivo in the rhesus monkey, [^{14}C]-hydrocortisone, with and without salicylic acid, was applied in acetone and the solvent evaporated. The compounds also were applied in a formulation (60% ethanol, 5% propylene glycol, 5% glycerin, 30% water) in which salicylic acid enhanced penetration in vitro (18). There was a difference in the kinetics of hydrocortisone absorption with the two formulations. In acetone, excretion of ^{14}C in urine peaked at 48 hr and then declined. With the other formulation, excretion peaked at 48 hr, remained relatively constant to 72 hr and then declined (Figure 5).

There was no statistical difference in the percutaneous absorption of hydrocortisone with the addition of salicylic acid (18).

The above study was done in the classical way by applying a single dose and then monitoring absorption. The study was historically done before we investigated the absorption of hydrocortisone during chronic administration (16). This brings up the intriging hypothesis that perhaps if chronic application of hydrocortisone alters the penetration barrier and enhances its own absorption, would it not also enhance the penetration of a co-administered compound such as salicylic acid? This study has not been done.

With salicyclic acid there is another interesting study done recently by Roberts and Horlock (19). They examined the effect of repeated skin application of salicylic acid on its own percutaneous absorption. With chronic administration of salicylic acid they saw a significant increase in penetration flux during the first five days of application. This is the same response seen with daily application of hydrocortisone (16). Perhaps with daily co-administration of both salicylic acid and hydrocortisone, both compounds have enhanced penetration and this explains the enhanced clinical efficacy.

It should be pointed out that Roberts and Horlock (19) did see a reduction in penetration flux with weekly application. They suggested that a broadening in the stratum corneum may have accounted for this. What this means to the above hypothesis is speculation. However, the fact that they took a new approach to skin

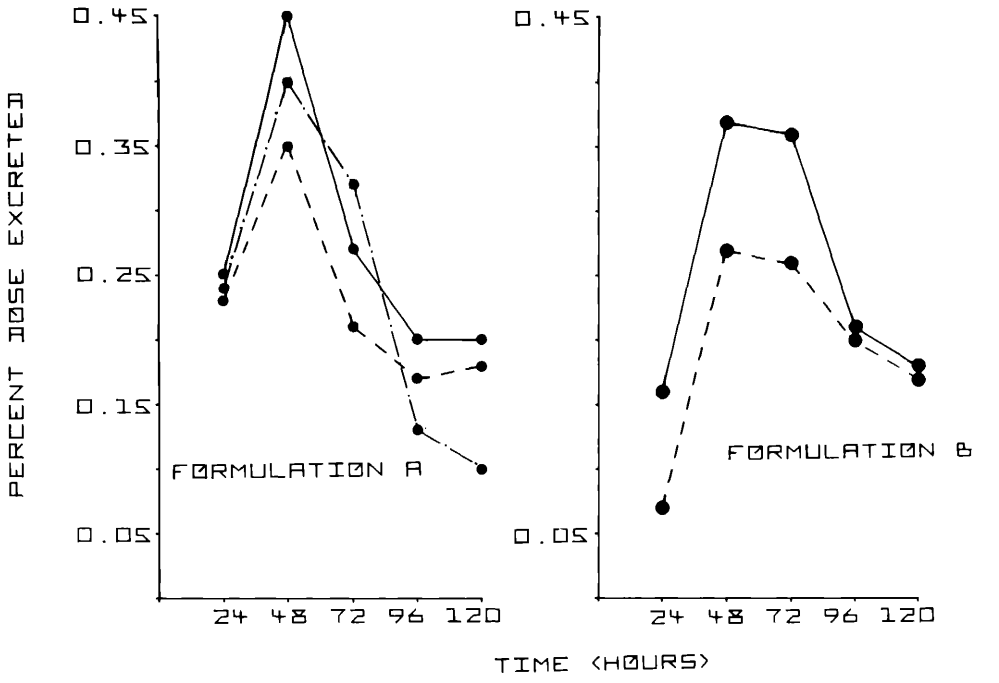

Figure 5. Kinetics of percutaneous absorption of hydrocortisone with and without addition of salicylic acid. Solid line indicates hydrocortisone 13.3 $\mu g/cm^2$; dash-dotted line, hydrocortisone, 13.3 $\mu g/cm^2$ and salicylic acid, 13.3 $\mu g/cm^2$; dashed line, hydrocortisone, 13.3 $\mu g/cm^2$ and salicylic acid, 133.3 $\mu g/cm^2$; Formulation A is acetone. Formulation B is ethanol (60%), propylene glycol (5%), glycerin (5%), and water (30%). Reproduced from Wester, Noonan and Maibach (15) with the permission of the American Medical Association.

penetration (chronic application) to help explain the clinical efficacy seen with salicylic acid is encouraging.

PERCUTANEOUS ABSORPTION IN THE NEWBORN

Percutaneous absorption of testosterone was determined in the newborn rhesus monkey (20). Mean percentage of absorptions of 4 and 40 $\mu g/cm^2$ in the newborn were, respectively, 22.5 ± 2.2 (SD) and 6.8 ± 2.1. Statistical comparisons (student's t-tests) of these results with those obtained with adults show no significant difference ($P > 0.05$) in skin penetration of testosterone in newborn and adult rhesus monkeys (Figure 6). With one other newborn rhesus, a topical dose of 40 $\mu g/cm^2$ was applied to the ventral forearm and the area was occluded for 24 hr. Percutaneous absorption was 14.7%, a value twice that from nonoccluded absorption.

Skin permeability in the newborn infant is an important concern because of the possible toxicity which could result from this route of drug delivery. A high percentage of a compound can be absorbed through the skin of a newborn. With the newborn there is another pharmacokinetic parameter, body weight, to also consider. Once the compound (and/or metabolites) is absorbed, it is available systemically. In the newborn the ratio of surface area (square centimeters) to body weight (kilograms)

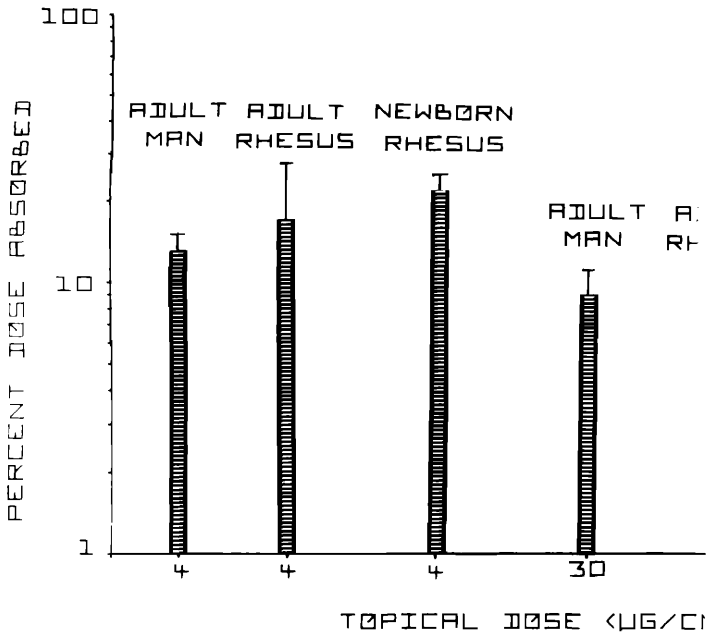

Figure 6. Comparison of percutaneous absorption of testosterone in newborn rhesus, adult rhesus, and adult man. The black bars show the percentage of dose absorbed (and standard deviation) per topical dose (micrograms per cm^2) applied. There is no statistically significant difference in the absorption of newborn and adult rhesus. Reprinted from Wester, Noonan, Cole and Maibach (20) with permission of the International Pediatric Research Foundation, Inc.

is three times that of the adult. Therefore, given equal application area of skin per newborn and adult, the systemic absorption seen in the newborn can be much more when based on body weight. As an example, if 0.1 g of a compound is applied to the total skin surface of an adult who weighs 70 kg, and 20% of the dose is absorbed, them:

Systemic availability (mg/kg)

$$= \text{dose (mg)} \times \% \text{ absorbed/body weight (kg)}$$
$$= 100 \text{ mg} \times 0.2/70 \text{ kg} = 0.28 \text{ mg/kg}$$

The surface area of a newborn is 2,200 cm^2, or 13% of that of an adult (17,000 cm^2). Applying the same strength compound to the total surface of a newborn would take only 13% of the 0.1 g, a topical dose of 13 mg. Given the same percutaneous absorption (20%), then in a newborn weighing 3.4 kg:

$$\text{Systemic availability (mg/kg)} = 13 \text{ mg} \times 0.2/3.4 \text{ kg} = 0.76 \text{ mg/kg}$$

Therefore, by topically applying the same strength compound to both the adult and the newborn, the systemic availability in the newborn is 2.7 times that of the adult. With a different ratio of skin surface to body weight, the therapeutic ratio probably is lower in the newborn than in the adult when the compound is applied topically. This increased systemic availability in the newborn would also be interrelated with any differences in systemic metabolism between the newborn and the adult.

SUMMARY

We have attempted to examine an animal model which would be more relevant to man than the animals used in the past to determine percutaneous absorption of compounds. We have an animal model, the rhesus monkey, in which some absorption data was comparable to man. We realize that the data are limited to the few comparative studies reviewed, and therefore the animal model must still be assumed limited and experimental in nature. The results are encouraging enough to warrant continued use of this animal model when the opportunity is available.

We have attempted to design percutaneous absorption studies which we believe to be more relevant to the clinical situation than the classical procedures used previously. Our results at times have been as expected, and at other times the results have been quite different than what was expected. However, these are first attempts in new areas of endeavor and they should hopefully provoke new interest in explaining percutaneous absorption. Future studies in both animals and man with new approaches in experimental design will lead to results which will impact greatly in the toxicology and clinical efficacy of topical compounds.

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