Methods for *In Vitro* Percutaneous Absorption Studies III: Hydrophobic Compounds

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Received May 9, 1983, from the *Division of Toxicology, Food and Drug Administration, Washington, DC 20204.* Accepted for publication September 8, 1983.

Abstract □ The absorption of two hydrophobic compounds through rat skin was measured by *in vivo* and *in vitro* techniques. The permeation of the fragrance ingredients 3-phenyl-2-propenyl 2-aminobenzoate (I) and 1-(3-ethyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethanone (II) was measured from a petrolatum and an acetone vehicle. Increases in permeation of 8-fold (I) and 95-fold (II) were observed when the compounds were tested *in vivo* under conditions similar to *in vitro* procedures. The apparent inability of the compounds to freely enter the diffusion cell receptor fluid was partially reversed by replacing normal saline with other fluids: rabbit serum, 3% bovine serum albumin, organic solvents, and dilutions of four nonionic surfactants. The effect of the receptor fluids on the integrity of the skin barrier was assessed by measuring the permeability of control compounds (cortisone, urea, and water). A 6% solution of polyethylene glycol 20 oleyl ether was the receptor fluid of choice. Without apparent damage to the skin, 61% (petrolatum vehicle) or 73% (acetone vehicle) of the *in vivo* absorption of I was obtained. With II, only 32% of the *in vivo* absorption was achieved (petrolatum vehicle). Even when the surfactant solution is used, significant differences may still remain between *in vivo* and *in vitro* results.

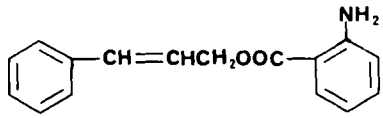
Keyphrases □ Absorption—percutaneous, *in vitro*, hydrophobic compounds □ Hydrophobic compounds—*in vitro* percutaneous absorption

For a number of compounds, good agreement is found between the results of *in vivo* and *in vitro* skin permeability studies (1-10). The *in vitro* values determined by Franz (1, 2) compared favorably with the *in vivo* permeability results of Feldmann and Maibach (3) for 12 organic compounds in human skin when considerations were made for experimental differences. Bronaugh *et al.* (4) have measured both the *in vivo* and *in vitro* absorption of three compounds through rat skin. Again, the diffusion cell studies were accurate in their prediction of *in vivo* absorption.

For a certain type of compound, however, erroneous results may be obtained when standard diffusion cell techniques are used. Compounds that are essentially insoluble in water may not partition freely from excised skin into an aqueous receptor fluid. When applied to skin *in vivo*, however, these compounds may readily leave the barrier layers of the skin because of their solubility in biological fluids. In the *in vivo* and *in vitro* absorption comparisons of Tsuruta (8, 9), values much lower than expected were obtained for *in vitro* permeability measurements of organic solvents with the lowest water solubility.

The problem was alluded to by Franz (1), who in selecting compounds for study, omitted highly water-insoluble compounds to avoid results that were "artificially limited due to insolubility in the dermal bathing solution." Brown and Ulsamer (11) have found that the skin permeation of the hydrophobic compound hexachlorophene increased twofold when normal saline was replaced with 3% bovine serum albumin (in a physiological buffer) in the diffusion cell receptor. This new receptor fluid had a 500-fold greater solubility for hexachlorophene. In a recent study (12), a nonionic surfactant (poloxamer 188[1]) was used to enhance the solubility of linoleic acid in the diffusion cell receptor medium. Although the potential problem inherent in the partitioning of a hydrophobic compound from skin is now recognized, no systematic studies to characterize and attempt to solve this problem have been carried out.

In determining the skin absorption of two fragrance materials, it seemed that their hydrophobic nature might cause difficulties in an *in vitro* analysis. The water solubilities of 3-phenyl-2-propenyl 2-aminobenzoate (cinnamyl anthranilate; I) and 1-(3-ethyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethanone (acetyl ethyl tetramethyl tetralin; II) were 0.23 and 0.012 mg/L, respectively. Therefore, percutaneous absorption of these compounds was initially determined *in vivo* in rats. Lipophilic receptor fluids were substituted for normal saline to try to enhance absorption and approximate the *in vivo* results without damage to the permeability barrier. Rabbit serum and aqueous dilutions of serum albumin, organic solvents, and four commonly used nonionic surfactants were utilized as the lipophilic receptor fluids.



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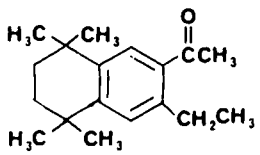

II

EXPERIMENTAL SECTION

Materials—The following radiolabeled compounds were used to facilitate quantitation of skin absorption (specific activity): [^{14}C]II[2] (2.40 mCi/mmol); [^{14}C]I[2] (10.5 mCi/mmol); [^{3}H]cortisone[4] (43.0 Ci/mmol); [^{14}C]urea[4] (57.0 mCi/mmol); and tritiated water (55.5 mCi/mmol). All compounds were determined to have a radiochemical purity of 98%. Agents used in the receptor fluids, polyethylene glycol 20 oleyl ether (PEG-20 oleyl ether)[5], octoxynol 9[6], poloxamer 188, polysorbate 80[7], and bovine serum albumin[8], were prepared in distilled water at the indicated concentrations. All agents (except albumin) are commonly used nonionic surfactants.

***In Vitro* Studies**—Percutaneous absorption measurements were made in a one-chambered static diffusion cell as described previously (13). The receptor fluid was stirred with a magnetic stirring bar in a constant-temperature water bath maintained at 32°C.

The skin on the back was lightly shaved and then excised from 3- to 7-month-old female Osborne-Mendel rats. In some experiments, subcutaneous tissue was removed, and full-thickness skin was fixed in the diffusion cell. For most experiments, split-thickness skin (350 μm) was prepared with a dermatome[9]. Test compounds were applied to the epidermal surface of the skin

[1] Pluronic F-68; BASF-Wyandotte, Wyandotte, Mich.

[2] Arthur D. Little, Cambridge, Mass.

[3] Division of Cosmetics Technology, Food and Drug Administration, Washington, D.C.

[4] New England Nuclear Corp., Boston, Mass.

[5] Volpo 20; Croda, Inc., New York, N.Y.

[6] Triton X-100; Rohm and Haas Co., Philadelphia, Pa.

[7] Tween 80; Fisher Scientific Co., Fair Lawn, N.J.

[8] Sigma Chemical Co., St. Louis, Mo.

[9] Padgett Dermatome, Kansas City, Mo.

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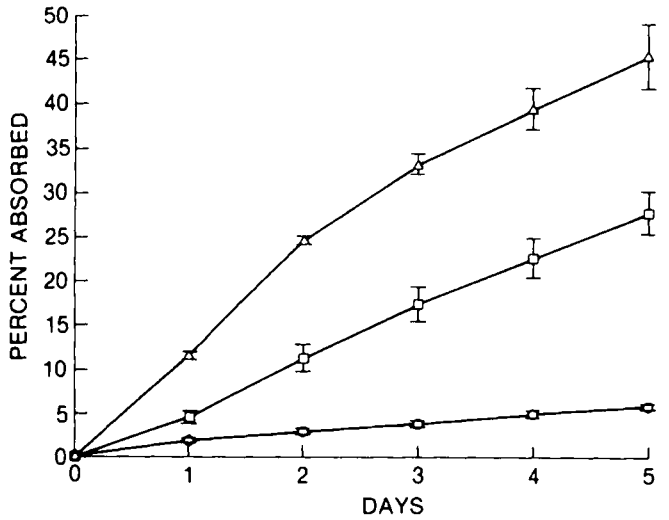

Figure 1—*Absorption of I from a petrolatum vehicle. Key: (Δ) in vivo results; (○) in vitro (saline in receptor); (□) in vitro (6% PEG-20 oleyl ether in receptor). At 5 d, 13% of the in vivo absorption was obtained by in vitro techniques, using saline in the diffusion cell receptor. Absorption was increased to 61% of the in vivo results by substituting 6% PEG-20 oleyl ether as the receptor fluid.*

dissolved in either a petrolatum or acetone vehicle. The 1.13-cm^2 area of skin was completely covered by the vehicle (30 mg of petrolatum, 20 μL of acetone). Aliquots of receptor fluid were removed from a side arm at various intervals, and the radioactivity was determined in a liquid scintillation counter[10].

Cortisone permeation was determined to monitor the effect of the receptor fluid on the integrity of the skin barrier. In most experiments, the permeability of the ^{14}C-labeled fragrance and the [^3H]cortisone was determined simultaneously by dual-label techniques. The absorption of the fragrances (I and II) was expressed as a percentage of the amount applied to the skin to facilitate comparison with the *in vivo* results. Cortisone permeation from the petrolatum vehicle was expressed as a permeability constant (steady-state absorption rate divided by the applied concentration). Other percutaneous absorption values were expressed as the percent dose absorbed and/or permeability constants.

***In Vivo* Studies**—The skin absorption of I and II was determined in rats by *in vivo* methods described previously (4). The back skin of the rats was lightly clipped and a nylon ring (inside area, 2 cm^2) was glued to the back with a cyanoacrylate adhesive. The rats were partially restrained with rubber tube collars, and the test compounds were applied inside the protective device on the back. Because of significant excretion in the feces expected with the lipophilic compounds, absorption was estimated by summing the amounts of compound excreted in the urine and feces (14). These samples were collected at 24-h intervals in metabolism cages for 5 d. Radioactivity in the samples was determined by liquid scintillation counting. Percutaneous absorption was expressed as the percentage of the applied dose excreted during the collection periods.

Statistical analysis was by the Student's *t* test. The significance of increased absorption of compounds resulting from changes in the receptor fluid was determined by using a one-tailed probability.

RESULTS

In vitro percutaneous absorption measurements, using a normal saline receptor solution, resulted in values that were much lower than the corresponding *in vivo* data for I and II. This difference is shown in the time course for the absorption of I (Fig. 1). In initial studies, the effect of different receptor fluids on the absorption of I was determined by comparing values obtained after 5 d (Table I). Only 12.7% of I absorbed *in vivo* during the 5-d period was obtained in a corresponding *in vitro* experiment, using normal saline receptor fluid and split-thickness rat skin. A 1.5% solution of the nonionic surfactant PEG-20 oleyl ether enhanced by threefold the skin permeability to I; the cortisone permeation determined simultaneously in the dual-label experiment was not altered. When full-thickness rat skin was used, no increase in the absorption of I was obtained with the PEG-20 oleyl ether. The optimal concentration of the surfactant was 6%. A fivefold increase in the absorption of I was obtained without altering the permeation of the cortisone control.

Rabbit serum and bovine serum albumin had no effect on the integrity of the barrier, but they were also less effective in increasing the absorption of I than PEG-20 oleyl ether. A methanol–water solution and 6% octoxynol 9 were equal or superior to 6% PEG-20 oleyl ether, but significant damage to the skin was indicated by the increased cortisone permeation since the absorption of cortisone is not limited by a lack of solubility in the normal saline receptor fluid. A 6% solution of poloxamer 188 in the receptor resulted in slight enhancement of both I and cortisone permeation.

An even greater difference was seen between the *in vivo* absorption of II and the diffusion cell data (Table I). Results similar to those determined with I were obtained by altering the receptor fluid. The enhanced absorption with 1.5% PEG-20 oleyl ether required the use of a dermatome skin section. Best results were obtained with 6% PEG-20 oleyl ether in the receptor, and no increase was observed in the permeation of the cortisone control. Use of a glycerol–water solution in the receptor resulted in permeability data similar to those obtained with normal saline. As was previously found when a methanol–water solution was used, the ethanol–water receptor fluid enhanced the penetration not only of II, but also of the cortisone control.

When I was applied to rat skin in an acetone vehicle, greater absorption was obtained than with a petrolatum vehicle (Table II). A 6% PEG-20 oleyl ether receptor fluid in the diffusion cells greatly increased the permeation of I without a significant effect on the permeation of cortisone.

The *in vitro* absorption of II and cortisone (acetone vehicle) was measured for 43 h to determine the effect on skin permeation of three nonionic surfactants (Table II). Use of 6% PEG-20 oleyl ether resulted in the greatest increase in the absorption of II without an increase in that of the cortisone control. At the same concentration (6%), both polysorbate 80 and octoxynol 9 resulted in increased cortisone permeation when compared with the value obtained with normal saline.

The effect of 6% PEG-20 oleyl ether on the absorption of water and urea

Table I—Effect of Diffusion Cell Conditions on the Absorption of I and II (Cortisone Control)[a]

Receptor Fluid	Percent Applied Dose Absorbed in 5 d	Cortisone Permeability Constant × 10^5
	Compound I	
Normal saline (4)[b]	5.0 ± 0.3	3.8 ± 0.7
1.5% PEG-20 oleyl ether (4)[b]	5.4 ± 0.9	–
Normal saline (4)	5.8 ± 0.4	7.1 ± 0.5
1.5% PEG-20 oleyl ether (10)	15.5 ± 1.2[c]	6.1 ± 0.5
6% PEG-20 oleyl ether (8)	27.9 ± 1.8[c,d]	7.0 ± 0.9
20% PEG-20 oleyl ether (8)	18.3 ± 1.8[c]	9.3 ± 0.9
Rabbit serum (4)	8.8 ± 0.6[c]	6.8 ± 0.8
3% Bovine serum albumin (4)	12.1 ± 1.2[c]	5.4 ± 0.2
50:50 Methanol–water (4)	27.1 ± 2.0[c]	17.2 ± 0.2[e]
1.5% Octoxynol 9 (4)	17.9 ± 1.1[c]	10.8 ± 0.5[e]
6% Octoxynol 9 (4)	38.4 ± 2.9[c]	14.5 ± 1.3[e]
6% Poloxamer 188 (4)	7.3 ± 1.3	9.8 ± 0.6[e]
	Compound II	
Normal saline (6)[b]	0.08 ± 0.01	3.8 ± 0.7[e]
1.5% PEG-20 oleyl ether (6)[b]	0.24 ± 0.07	–
Normal saline (4)	0.20 ± 0.06	6.3 ± 0.3
1.5% PEG-20 oleyl ether (4)	2.3 ± 0.4[c]	4.9 ± 0.2
6% PEG-20 oleyl ether (4)	6.0 ± 0.9[c,f]	7.0 ± 0.9[e]
50:50 Glycerol–water (3)	0.14 ± 0.03	4.7 ± 0.9
40:60 Ethanol–water (4)	6.1 ± 1.2[c,g]	21.7 ± 3.3[e]

[a] Values are the mean ± *SE*; the number of determinations is in parentheses. For most experiments, a 350-μm section from the surface of whole rat skin was prepared with a dermatome. Compounds were applied to the skin in a petrolatum vehicle. *In vivo* absorption of I was 45.6%; *in vivo* absorption of II was 18.9%. [b] Whole skin. [c] Significant increase when compared with results from the appropriate saline control (dermatome section) by one-tailed Student's *t* test, $p < 0.05$. [d] Significant increase when compared with results from all receptor fluid for I except methanol–water and 6% octoxynol 9 by one-tailed Student's *t* test, $p < 0.05$. [e] Value determined in experiments with I. [f] Significant increase when compared with results from all receptor fluids for II except ethanol–water by one-tailed Student's *t* test, $p < 0.05$. [g] Value determined at 4 d.

[10] Model LS 9000; Beckman Instruments, Fullerton, Calif.

Table II—Effect of Nonionic Surfactants on I, II, and Cortisone Absorption[a]

Receptor Fluid	Percent Absorbed (5 d) I	Cortisone
Compound I[b]		
Normal Saline (9)	14.1 ± 1.3	21.5 ± 2.5
6% PEG-20 oleyl ether (8)	44.1 ± 4.3[c]	24.8 ± 3.2
Compound II		
Receptor Fluid	Percent Absorbed (43 h) II	Cortisone
Normal Saline (12)	0.7 ± 0.1	7.2 ± 0.9
6% PEG-20 oleyl ether (8)	7.5 ± 1.4[c]	6.0 ± 0.5
6% Polysorbate 80 (4)	4.1 ± 0.6[c]	10.6 ± 1.5[c]
6% Octoxynol 9 (4)	11.9 ± 1.8[c]	15.1 ± 1.0[c]

[a] Values are the mean ± *SE*; the number of determinations is in parentheses. Compounds were applied *in vitro* to rat skin (350-μm section) in an acetone vehicle. [b] *In vivo* absorption of I (acetone vehicle) was 60.7%. [c] Significant increase when compared with the appropriate saline control by one-tailed Student's *t* test, $p < 0.05$.

was examined with rat skin in diffusion cells (Table III). No significant increase was seen in the percutaneous absorption of these two additional control compounds.

DISCUSSION

A comparison of the *in vivo* and *in vitro* percutaneous absorption data for the fragrance ingredients I and II reveals the underestimation of absorption that can occur by using diffusion cell techniques with hydrophobic compounds. Increases of 8-fold (I) and 95-fold (II) in permeation were obtained when the compounds were tested *in vivo* under similar conditions.

In vitro absorption was therefore increased by substituting more lipophilic receptor fluids for normal saline. A physiological-based solution to the problem, it seemed, might result from the use of serum or serum proteins in the diffusion cell receptor. However, the use of rabbit serum as the receptor fluid did not substantially increase absorption of the fragrances. This failure may be the result of the absence of a continual replacement of the bathing medium, as occurs *in vivo* with blood flow through the skin. Use of 3% bovine serum albumin was more effective than serum, but only about a twofold increase in I absorption was obtained. Brown and Ulsamer (11) have observed a similar increase in hexachlorophene permeation through excised human stratum corneum when 3% bovine albumin was added to the receptor. The penetration rate of hexachlorophene correlated with the solubility of the compound in different buffer-albumin mixtures.

Nonionic surfactants have a relatively mild effect on skin, with a much lower irritation potential than ionic agents (15). Aqueous dilutions of a series of nonionic surfactants were evaluated in the diffusion cell system. The fact that split-thickness skin was required for an increase in I and II absorption with 1.5% PEG-20 oleyl ether indicates that a more physiological value may be obtained with this preparation of skin. A major portion of systemic uptake occurs in the highly vascular upper dermis; thus, use of full-thickness skin *in vitro* can introduce an artificial barrier, which is of importance particularly with hydrophobic compounds.

More than 60% of the absorption of I that occurred *in vivo* was obtained by using diffusion cells with a 6% solution of PEG-20 oleyl ether (Table I). The permeability constant for cortisone was unaffected. Attempts to increase the absorption of I with a 20% solution of the surfactant resulted in a slight increase in cortisone permeation and an unexpected decrease in absorption of I. The high viscosity of a 20% solution makes it difficult to use experimentally. Solutions of octoxynol 9, poloxamer 188, and polysorbate 80 resulted in enhanced permeation of the cortisone control in experiments with I and II, even at concentrations at which lower amounts of these hydrophobic compounds were absorbed than with the PEG-20 oleyl ether (Tables I and II). A 6% solution of PEG-20 oleyl ether was found to be the receptor fluid of choice.

Table III—Effect of PEG-20 Oleyl Ether on the Absorption of Water and Urea[a]

Receptor Fluid	Water Permeability Constant × 10^3	Percent Absorbed (43 h) Water	Urea
Normal Saline	2.5 ± 0.6	21.0 ± 5.4	9.3 ± 3.2
6% PEG-20 oleyl ether	2.7 ± 0.6[b]	23.5 ± 5.5[b]	10.3 ± 3.4[b]

[a] Values are the mean ± *SE* of eight determinations. Compounds were applied to rat skin (350-μm section) in 600 μL of water. [b] No significant increase when compared with saline controls by one-tailed Student's *t* test, $p < 0.05$.

Table IV—Comparison of Solubility Properties and the Effect of PEG-20 Oleyl Ether on Percutaneous Absorption

Compound	Water Solubility, mg/L	Octanol-Water, K	Skin Permeation Ratio[a]
Urea	1×10^6	0.002	1.1[b]
Cortisone	280	44	1.2[c]
Testosterone	11	2089	2.3[d]
I	0.23	652	3.1[c]
II	0.012	3589	30.0[e]

[a] 6% PEG-20 oleyl ether/saline. The skin permeation ratio was determined by comparing the amount of compound absorbed in experiments in which the two receptor fluids were used. [b] Water vehicle, 43 h. [c] Acetone vehicle, 5 d. [d] Acetone vehicle, 43 h. [e] Petrolatum vehicle, 5 d.

The use of cortisone as a control in many of the experiments was based on its good solubility in the petrolatum vehicle, reasonable water solubility, and ready availability in the tritiated form for dual-label experiments. It was considered important to establish additionally that the permeation of other molecules was also unaffected by the use of 6% PEG-20 oleyl ether as a receptor fluid. The percutaneous absorption of two small polar molecules (water and urea) was not significantly increased by the use of the surfactant solution (Table III).

The use of an acetone vehicle resulted in better agreement between the *in vitro* data for I and the diffusion cell results with PEG-20 oleyl ether as the receptor fluid (Table II). *In vitro* permeation of I after 5 d was 73% of that found in the corresponding *in vivo* experiment. This result may be due to higher levels of I absorbed into the skin in the absence of the lipophilic petrolatum layer.

The increase in absorption resulting from the use of 6% PEG-20 oleyl ether instead of saline in the receptor is presented in Table IV along with the octanol and water solubility properties of selected compounds. No significant effect of using PEG-20 oleyl ether was obtained with the more water-soluble compounds. The greatest increase in permeation was obtained with II, an extremely hydrophobic compound with a large octanol-water partition coefficient. Compounds that are essentially insoluble in water (solubility, ~10 mg/L or less) and more soluble in lipoidal media may partition only slightly from skin into a normal saline solution. For these types of compounds, caution must be exercised in interpreting *in vitro* absorption data.

In a system in which split-thickness rat skin is used as the membrane, the use of a surfactant in the receptor fluid was advantageous for absorption measurements of several hydrophobic compounds. Although results identical to *in vivo* values were not obtained, dramatic increases in absorption were achieved without apparent damage to the skin. Obviously, one receptor fluid is not beneficial to the same degree for all hydrophobic compounds. The permeation of compounds like II, which are extremely lipophilic and hydrophobic, will be increased to the greatest extent, but even so, <50% of the *in vivo* absorption may be obtained. For compounds with a lower lipid-water partition coefficient, values more closely in agreement with *in vivo* results should be achieved.

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Kinetics of Aspirin Absorption Following Oral Administration of Six Aqueous Solutions with Different Buffer Capacities

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Received October 29, 1982, from the *Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri-Kansas City, Kansas City, MO 64108.* Accepted for publication September 13, 1983.

Abstract □ Fifteen volunteers each received two of six aspirin solutions in a balanced incomplete block design. The solutions contained 0, 3, 6, 10, 16, and 34 mEq of sodium bicarbonate-citric acid buffer and 650 mg of aspirin. Plasma aspirin levels were measured in blood samples collected frequently during the first 2.5 h, and the accumulation of aspirin, salicylic acid, and salicyluric acid were measured over 2 and 24 h. The most rapid absorption rates occurred with solutions which contained small quantities of the antacid buffer. The 3- and 6-mEq antacid buffers had mean maximal aspirin concentrations of 17.3 and 17.8 μg/mL, respectively. In the absence of the buffering agent, the 650 mg of aspirin failed to dissolve completely and gave a mean maximal plasma concentration of 13.4 μg/mL. With 34 mEq of a buffering agent, a delay in the onset of absorption occurred and the presystemic hydrolysis increased. This was probably because more aspirin was emptied into and absorbed from the small intestine with higher concentrations of the buffering agent.

Keyphrases □ Aspirin—pharmacokinetics, oral administration, sodium bicarbonate-citric acid buffer, humans □ Pharmacokinetics—aspirin, sodium bicarbonate-citric acid buffer, oral administration, humans

Aspirin, the drug of choice when a mild analgesic-antipyretic is required, is also the primary agent in the chronic management of rheumatoid arthritis and osteoarthritis. After oral administration, rapid absorption beneficially provides rapid onset of effects and reduces the contact time with the gastric mucosa.

In an earlier report from this laboratory (1) the literature relating to the administration of aspirin in buffered solutions was reviewed, and data comparing an unbuffered tablet to two different buffered solutions was presented. The three dosage forms were an unbuffered tablet, an effervescent solution with 16 mEq of buffer, and an effervescent solution with 34 mEq of buffer. Significant differences in the aspirin absorption rate were observed: the solution of 16 mEq of buffer was fast, the solution of 34 mEq of buffer was intermediate, and the unbuffered tablet was slow. These differences were attributed primarily to gastric emptying and the rate of tablet dissolution.

It may be desirable to optimize the amount of buffer components to provide rapid absorption and to reduce gastric irritation through minimal alkalinization of urine and sodium intake. Thus, this study was undertaken to evaluate aspirin absorption kinetics over a range of buffer strengths.

EXPERIMENTAL SECTION

Dosage Forms—A series of soluble tablets were prepared that contained 650 mg of aspirin and sufficient sodium bicarbonate-citric acid buffer to provide 0, 3, 6, 10, 16, and 34 mEq of buffering. Just prior to administration, the tablets were placed in 140 mL of water and mechanically stirred to effect dissolution. The unbuffered tablet contained some undissolved particles in suspension, while all buffered tablets dissolved completely. Immediately following administration, the containers were rinsed with 100 mL of water which was also swallowed by the volunteers.

Subjects—Fifteen healthy volunteers, 21–33 years old and weighing 56.4–81.8 kg, were screened by a comprehensive physical examination, complete blood chemistry, complete blood count with differential, and urinalysis. All were free of any active disease such as influenza, and denied any use of medication for 14 d prior to the study. None of the subjects had a history of GI disease or surgery.

Method—The balanced incomplete block design, Table I, in which each of the 15 subjects received two of the six dosage forms with a 6-d washout between treatments, was employed. A 10-h fast preceeded dosing and continued 4 h postdose, except for water. Predose urine and blood samples were obtained, and a single dose of aspirin (650 mg) with 240 mL of water was administered as described. After dosing, 100 mL of water was administered at 1-, 2-, and 3-h intervals, and a uniform meal was served after 4 h. Subjects remained standing or sitting throughout the first 4 h, and exercise was limited to walking about the room.

Blood was drawn into chilled vacuum containers[1] *via* an indwelling catheter at 5, 10, 15, 20, 30, and 45 min and at 1, 1.5, 2, and 2.5 h. Plasma was separated by centrifugation at 1764×*g* within 20 min of collection. All urine was collected over a 0–2-h interval, the pH and volume were measured, and a 4-mL aliquot was taken for analysis. In addition, all urine was collected for 24 h. All plasma and urine samples were handled as described previously (2), which included storage at −20°C and HPLC analysis within 2 weeks. The HPLC method was updated by using a 5-μm octadecasilane column[2], measuring absorbance at 237 nm, and replacing the benzene with butyl chloride. These changes improved the sensitivity, safety, and reproducibility of the method over that reported previously (2); the statistics of the revised method will be the subject of a subsequent report.

Plasma aspirin concentration *versus* time plots for each dose to each subject were evaluated, and estimates for several kinetic parameters were obtained. The highest observed plasma concentration (C_{max}) and the time after dosing (t_{max}) to reach C_{max} were read directly from each plot. A terminal rate constant (k) and corresponding half-life ($t_{1/2}$) were computed by linear regression analysis of the natural logarithm of plasma concentration *versus* the time for all times greater than twice t_{max}. The trapezoid method was used to compute the area under the curve to the last measured concentration (C) and the value

[1] Vacutainer BD, 278-069, 7.0-mL capacity containing 14 mg of potassium oxalate and 17.5 mg of sodium fluoride.
[2] Altex Ultrasphere, ODS 5 μm; Altex Scientific Inc., Berkeley, Calif.

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