Table I—Percentage of Nitroglycerin in Ointment Capsules Stored at 37° for 18 Weeks

Weeks	Percent Content (Mean ± *SE*, *n* = 5) Kinetic Assay	HPLC Assay
0	3.24 ± 0.06	3.19 ± 0.11
5	3.18 ± 0.10	3.23 ± 0.08
10	3.17 ± 0.12	3.20 ± 0.10
18	3.22 ± 0.05	3.19 ± 0.09

tions, the correlation coefficient of results obtained by the two methods was 0.988.

Unfortunately, the HPLC method and the other previously reported high-pressure liquid chromatographic method (10) were not specific for glyceryl dinitrates due to the presence of unknown interfering peaks from the ointment extract (Fig. 1). No reduction in size of the interfering peaks was achieved by using other organic solvents (hexane, ether, and methanol) to extract the ointment. The relative height of these peaks did not change over time. It was established by TLC separation (11) prior to HPLC analysis that the amount of glyceryl dinitrates present in the ointment was negligible. Triacetin, which is used as a solvent for nitroglycerin in some preparations, cochromatographed with glyceryl 1,2-dinitrate using the method of Crouthamel and Dorsch (8).

No loss of nitroglycerin was found after storage of individual capsules for 18 weeks at 37° (Table I). The amount of the dinitrates present in the ointment was negligible throughout the study.

The good stability of nitroglycerin in the ointment formulation tested is consistent with a high affinity of nitroglycerin for the lipophilic ointment base and a low affinity for the gelatin capsule in which the ointment is presented. In contrast, the availability of nitroglycerin from aqueous solutions infused from plastic infusion bags through giving sets is reduced due to the high affinity of nitroglycerin for the plastic (12).

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Paul A. Cossum
Michael S. Roberts ˣ
School of Pharmacy
University of Tasmania
G.P.O. Box 252C, Hobart
Tasmania, Australia

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Comparison of Plasma and Urine Analyses for Thiazides in Bioavailability/Bioequivalence Study

Keyphrases □ Thiazides—plasma *versus* urine analysis in bioavailability/bioequivalence study □ Bioavailability—thiazides, plasma *versus* urine analysis □ Bioequivalence—thiazides, plasma *versus* urine analysis □ Diuretics—thiazides, plasma *versus* urine analysis in bioavailability/bioequivalence study

To the Editor:

Thiazides are widely used as diuretics in the treatment of hypertension. In the recent proposal (1) for bioavailability/bioequivalence requirements, the thiazides were identified as having potential bioequivalence problems. To ensure bioequivalence of thiazide products, both *in vitro* dissolution and *in vivo* bioavailability requirements were proposed. The *in vivo* requirements call for bioavailability studies in humans and comparison of blood level and/or urinary excretion profiles of the drug with a standard reference product. It is generally assumed that blood (or plasma or serum) level measurements give a better assessment of bioavailability and bioequivalence than urinary measurements because of complicated pharmacokinetic considerations such as drug metabolism and urine collection problems.

It is sometimes argued that the presence of drug in the blood is not a real estimate of the drug availability at the site of action but only an estimate of drug bioavailability. It is preferable to determine the amount of drug at the site of action or to measure therapeutic effect, but these two procedures are generally not feasible. Thiazide diuretics are weak carbonic anhydrase inhibitors that enhance the renal excretion of sodium and chloride ions and an accompanying volume of water, thereby causing diuresis. This therapeutic effect is due to inhibition of ion reabsorption in the distal tubule. Thiazides are primarily excreted unchanged in urine by active secretion in the proximal tubule (2). Thus, from a pharmacological and a therapeutic viewpoint, measurement of the urinary excretion of the drug appears to be a logical choice in lieu of measurement in blood for the assessment of bioavailability of a diuretic dosage form.

Previous work on chlorothiazide and hydrochlorothiazide suggested that urinary level measurements give adequate information for bioavailability/bioequivalence assessment (3, 4). Figure 1 shows the amount of drug eliminated in the urine (AE) and the area under the curve (AUC) in the same subjects for various thiazides. For hydrochlorothiazide, ~34% of the administered drug was recovered in 0–12-hr urine samples at all dose levels (100, 50, and 25 mg), thus suggesting a dose–response relationship. As expected, the amount of drug in urine decreased with a decrease in hydrochlorothiazide administration, but the AUC did not correspond and correlate with the AE values (Subjects 2 and 3, Fig. 1A). Earlier data (5) for hydrochlorothiazide showed no relationship between the AUC and AE (Fig. 1B), between the AUC and product bioavailability, or between C_{max} and dose administered or bioavailability.

A preliminary study using chlorothiazide products showed that, although approximately the same amount of drug was excreted in 48 hr from subjects administered 250-

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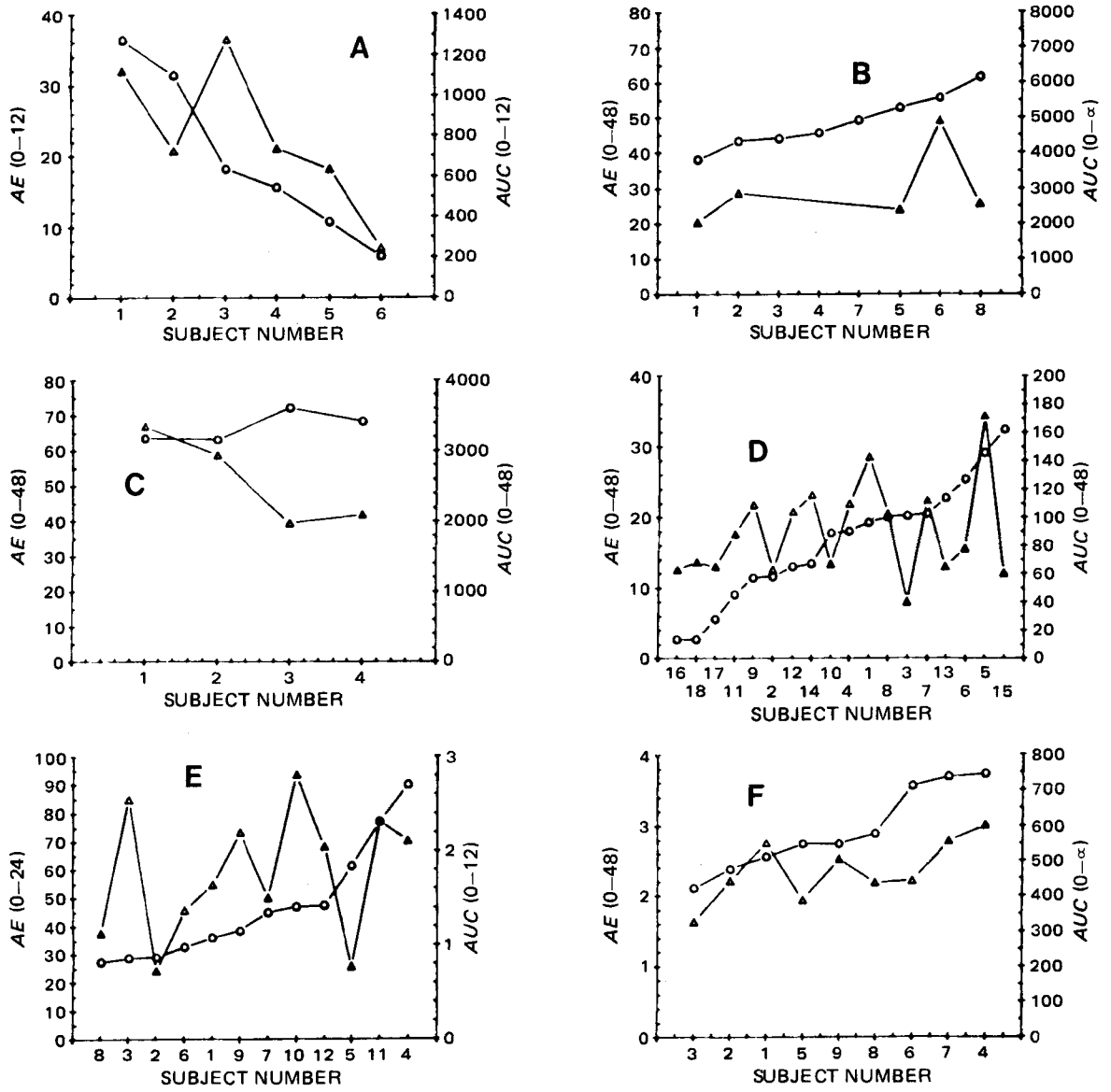

Figure 1—*Comparison of* AUC (△) *and* AE (○) *values for thiazides in the same subjects. Key: A, hydrochlorothiazide—Subjects 1 and 2 received a 100-mg dose, Subjects 3 and 4 received a 50-mg dose, and Subjects 5 and 6 received a 25-mg dose; B, hydrochlorothiazide—all subjects received a 75-mg dose; C, chlorothiazide—Subjects 1 and 2 received a 250-mg dose and Subjects 3 and 4 received a 500-mg dose; D, polythiazide—all subjects received a 1-mg dose; E, hydroflumethiazide—all subjects received a 100-mg dose; and F, bendroflumethiazide—all subjects received a 10-mg dose.*

and 500-mg doses, there was an approximate twofold difference in *AUC* (3), thus suggesting a lack of correlation between *AE* and *AUC* values (Fig. 1C).

Previous bioavailability studies involving plasma levels and urinary excretion measurements on polythiazide [Fig. 1D (6)], hydroflumethiazide [Fig. 1E (7)], and bendroflumethiazide [Fig. 1F (8)] were analyzed to determine the correlation between *AE* and *AUC* values. (The *AUC* values were calculated using the trapezoidal rule from the data provided.) Analysis of the data for the same panel of subjects shows wide variation in the *AUC* with nearly the same *AE*, suggesting a lack of correlation.

The lack of correlation observed between the plasma *AUC* and *AE* values is puzzling. One explanation may be the possible interaction of the thiazide with red blood cells. All thiazides have a sulfonamide group, and compounds with such groups have been shown to interact and bind with red blood cells (9). One hydrochlorothiazide study (10) also postulated an interaction between red blood cells and hydrochlorothiazide. The equilibrium drug concentration in plasma, which is most frequently measured for *AUC* determinations, is directly influenced by the interaction between the thiazide and red blood cells.

The reported plasma *AUC* measurements are more variable than the *AE* measurements, making it difficult to assess the amount of drug absorbed. On the other hand, the results of urinary excretion show comparatively less variation, making it easier for estimation of bioavailability/bioequivalence for thiazides. Thus, comparison of *AUC* and *AE* results indicate that urinary measurement from carefully planned and executed bioavailability studies are adequate to establish bioequivalence of thiazide diuretics.

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Vinod P. Shah ˣ
John P. Hunt
Vadlamani K. Prasad
Bernard E. Cabana
Biopharmaceutics Laboratory Branch
Food and Drug Administration
Washington, DC 20204

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Technique for Preparing Appendage-Free Skin (Scar) on Hairless Mouse

Keyphrases □ Scar tissue—technique for preparing appendage-free skin on the hairless mouse □ Burns—examination of scar tissue, technique for preparing appendage-free skin on the hairless mouse □ Skin—technique for preparing appendage-free skin on the hairless mouse, examination of scar tissue

To the Editor:

Scar formation is, minimally, a reconstruction of skin that has undergone total destruction of its epidermal elements through disease or physical trauma (1). Since epidermal elements such as hair follicles often penetrate deeply into and beyond the dermal structure, significant repair of dermal and subdermal damage also is associated with the appearance and function of the scarred surface. When cells of epidermal origin are obliterated within the surface, including those deep within the follicular invaginations, the epidermis of a small wound is repaired by lateral migration of cells from its periphery. Based on histological examination of actual injuries, an epidermal structure is formed, which, as a minimally functional renewed surface, is without normal skin lines and is devoid of pilosebaceous and eccrine appendages. It has a flattened interface with the dermis. The dermis is repaired by fibroblasts that appear in large numbers in the wound. Depending on the nature and extent of the damage, the scar may be raised or depressed and is usually schlerotic (hard) due to the synthesis of new collagen.

An appendage-free structure such as scar tissue should be useful for sorting out transfollicular and transepidermal contributions to percutaneous absorption by studying it side by side with normal skin. To do this study with the least possible complications, experimental wounds need to be developed that result in scarring but that also represent minimal damage and restructuring of the dermal matrix.

Many researchers have developed methods to produce experimental wounds in laboratory animals. These procedures include excision of surface tissues (2–7), branding (8–10), severe chemical burning (11), thermal burning (12), and X-ray and UV irradiation (13, 14). The major emphasis of these investigations has been to study the healing process; the end result, the scarred surface, has been of only incidental concern. Where scar tissue has been purposefully studied histologically and otherwise, tissue samples normally have been of etiological rather than of experimental origin (15, 16). Therefore, we attempted to develop a simple reliable technique to produce scars with minimal dermal involvement in small laboratory animals for the purpose of studying the physicochemical (barrier) properties of scar tissue itself.

Male hairless mice[1], 60–80 days old, were anesthetized with methoxyflurane[2], and their dorsal surfaces were scalded using a previously reported technique (17). Essentially, the dorsal surface of an animal was immersed for 30 sec in water maintained at 60°. These conditions were chosen because they produce a borderline full-thickness burn on hairless mouse skin (17) and are routinely used to separate epidermis from dermis in preparing epidermal membranes for mass transfer studies (18). At the outset, the conditions appeared to be at or near the threshold to accomplish the stated goal.

The scalded mice were placed on a table with the burned surfaces exposed. An area was marked on the traumatized dorsal surface roughly approximating 6–8 cm^2 of the body surface area. With the blade of a spatula, the epidermis was scraped off the surface. Occasionally, the epidermal layer was separated as a single piece, but it usually came off in fragments. The blade was moved back and forth on the exposed skin several times, exposing the outlines of superficial blood vessels. The skin was uniformly erythematous. The animals then were returned to individual

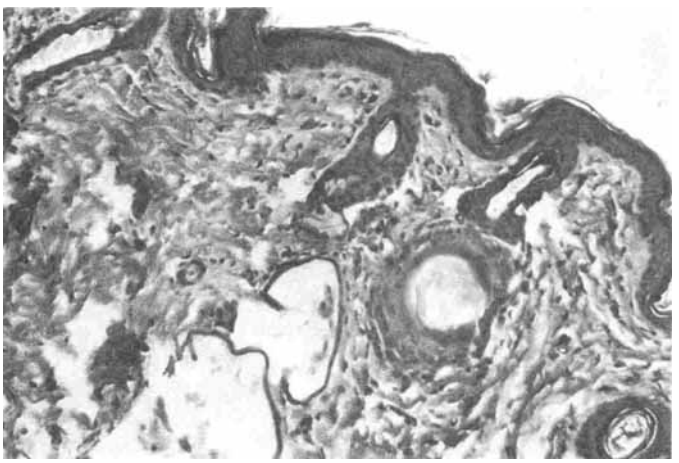

Figure 1—*Histological appearance of the hairless mouse normal skin.*

[1] SKH-hr^{-1} strain, Skin Cancer Hospital, Philadelphia, Pa.
[2] Metofane, Pitman-Moore, Washington Crossing, N.J.

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