Table I—Amount of Propranolol Represented by the Peak Concentration in Various Ocular Tissues [a]

Tissue	Peak Concentration, μg/g or μg/ml	Amount of Drug Present in Tissue, μg
Cornea	45.90	3.21
Aqueous humor	2.02	0.61
Iris	12.32	0.31
Lens	0.21	0.06

[a] The weights of the tissues are given in the text.

tissues. The approximate wet weight averages for the cornea, iris, aqueous humor, and lens in the current studies were 70, 25, 300, and 300 mg, respectively.

The relative disposition of propranolol in the various ocular tissues also was different than that of pilocarpine. There was a sixfold difference in peak concentration between the iris and the aqueous humor for propranolol, whereas the two tissues were virtually identical with pilocarpine (3). Furthermore, the penetration into the lens relative to other ocular tissues was greater for propranolol than for pilocarpine. Propranolol is known to act as a local anesthetic and as such may influence membrane permeability. It is unclear whether the differences in tissue distribution for propranolol and pilocarpine can be ascribed wholly, or in part, to this effect.

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Comparative *In Vitro* and *In Vivo* Antifungal Activity of Tolnaftate and Various Undecylenates

Keyphrases □ Antifungal activity—tolnaftate and various undecylenates, comparison *in vitro* and *in vivo* □ Tolnaftate—antifungal activity *in vitro* and *in vivo*, comparison with various undecylenates □ Undecylenates—antifungal activity *in vitro* and *in vivo*, comparison with tolnaftate

To the Editor:

Amsel *et al.* (1) compared the *in vitro* antifungal activity of undecylenic acid and tolnaftate and drew several conclusions. Among these conclusions were: (*a*) the undecylenate product killed the test organisms more rapidly than the tolnaftate-containing product; (*b*) undecylenates possibly are more effective in *in vitro* killing time than tolnaftate alone, and this finding probably applies to the commercial powders; and (*c*) although the concentrations of active ingredients varied in the commercial products tested, the undecylenates probably are more effective (as determined by killing time) than tolnaftate.

Table I—Agar Diffusion Study of Commercial Solutions T [a] and D [a] against Three Dermatophytes

Organism	Contact Time [b]	Zone Size, mm [c] T	D
T. mentagrophytes	1 min	30	0
	5 min	33	10
	15 min	31	11
	30 min	33	11
	1 hr	30	12
	4 hr	33	22
T. rubrum	1 min	42	± [d]
	5 min	20	±
	15 min	35	±
	30 min	41	±
	1 hr	37	±
	4 hr	38	17
E. floccosum	1 min	48	0
	5 min	50	0
	15 min	46	0
	30 min	50	5
	1 hr	47	10
	4 hr	45	10

[a] Commercial solution T contains 1% tolnaftate; commercial solution D contains 10% undecylenic acid. [b] Values between compounds were statistically significant ($p < 0.0001$) at all time points. [c] Using 6-mm disk. [d] These areas showed decreased mycelial growth but were not completely free of growth.

Table II—Agar Diffusion Study of Tolnaftate, Undecylenic Acid, and Zinc Undecylenate against Three Dermatophytes

Organism	Contact Time [b]	Zone Size after Incubation for 96 hr, mm [a] Tolnaftate Solution (0.1%)	Undecylenic Acid [c] Solution (0.1%)	Zinc Undecylenate [c] Suspension (0.1%)
T. mentagrophytes	1 min	29	± [d]	3
	5 min	34	±	8
	30 min	39	13	11
	1 hr	39	18	9
	3 hr	39	27	16
	6 hr	39	30	20
T. rubrum	1 min	43	±	±
	5 min	43	±	9
	30 min	44	18	9
	1 hr	47	25	15
	3 hr	47	33	26
	6 hr	47	>35 [e]	28
M. gypseum	1 min	28	0	0
	5 min	30	±	±
	30 min	37	±	6
	1 hr	38	14	±
	3 hr	39	23	17
	6 hr	39	25	20

[a] Using 6-mm disk. [b] Values between tolnaftate and undecylenates were statistically significant ($p < 0.0001$) at each time point. [c] Average of two disks. [d] These areas showed decreased mycelial growth but were not completely free of growth. [e] Too near edge of plate.

These conclusions were based on the methods, materials, and results of experiments presented in their paper. We have several concerns regarding their unusual methodology, and we therefore question their results and conclusions. We also wish to present *in vitro* and *in vivo* data using standard methods, which show, in contrast to the Amsel *et al.* (1) report, that tolnaftate is superior to the undecylenates. All data were submitted for statistical analysis using an appropriate analysis of variance, Duncan's multiple statistic test, and Fisher's exact test (2).

Our criticisms of the Amsel *et al.* (1) report are as follows:

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Table III—*In Vitro* Activity of Tolnaftate, Undecylenic Acid, and Zinc Undecylenate against Six Fungi in Sabouraud Dextrose Broth

Organism	Minimal Inhibitory Concentration, μg/ml					
	Tolnaftate[a]		Undecylenic Acid[b]		Zinc Undecylenate[a]	
	48 hr[c]	72 hr[c]	48 hr	72 hr	48 hr	72 hr
M. canis	0.055	0.3	7.5	7.5	7.5	7.5
M. gypseum	0.055	0.055	7.5	7.5	7.5	>10[e]
T. rubrum	0.055	0.055	3.75	7.5	3.75	7.5
T. mentagrophytes	<1.0[d]	<1.0	8.5	8.5	—	—
E. floccosum	<1.0	<1.0	8.5	8.5	—	—
A. niger	0.055	0.3	>10	>10	>10	>10

[a] Suspension in ethanol. [b] Solution in ethanol. [c] Values between tolnaftate and undecylenates were statistically significant ($p < 0.0001$) at 48 and 72 hr. [d] Lower levels not tested. [e] Higher levels not tested.

Table IV—Effect of Commercial Powders A[a] and C[a] against Topical *T. mentagrophytes* Infection in Guinea Pigs[b]

Compound	Number of Animals	Percentage of Cultures Negative		Average Lesion Score		Average Time until Animals Became Negative	
		During Treatment (10 days)	After Treatment (7 days)	During Treatment (10 days)	After Treatment (7 days)	Number of Animals	Day[c]
Commercial powder A	7	100[d]	100[e]	16.4[f]	3.9[g]	7	2[h]
Commercial powder C	7	0	33	18.2	6.1	4	15.5
						3	>17
Commercial talc	7	0	29	19.1	7.9	4	16
						3	>17
Untreated	5	0	7	18.1	7.0	1	17
						4	>17

[a] Commercial powder A contains 1% tolnaftate; commercial powder C contains 10% calcium undecylenate. [b] Treatment twice daily for 10 days. [c] First day of culture was Day 2. [d] Statistically significant ($p < 0.002$). [e] Statistically significant until Day 15 ($p < 0.05$). [f] Not statistically different. [g] Statistically significant ($p < 0.05$). [h] Statistically significant ($p < 0.002$).

1. Standard procedures for determination of the minimum fungicidal concentration in broth or agar were not used. In addition, the minimum inhibitory concentration for the various preparations used was not given for the test organisms, and the killing time results showed tremendous variation (up to 50%). If this new method is more reproducible than standard ones, all replicates should be the same since death was used as the end-point.

2. Tolnaftate was used as a suspension even though it is readily soluble (polyethylene glycol 400 or acetone) and solutions give more reproducible results than suspensions. In addition, the use of commercial powder A[1] as an aqueous suspension clearly is a weakly active mode of application that was never intended.

3. Polysorbate 20 is a surfactant used for emulsification, dispersion, and solubilization of lipids. Therefore, use of this substance makes the undecylenates more bioavailable since they are fatty acids, but it certainly is not useful for tolnaftate.

4. No evidence is presented that the complex rinsing solution and the washing procedure (only three washes) used for 2% undecylenic acid and 1% tolnaftate (where no differences were seen between the two drugs) were sufficient to wash out both the 20% zinc undecylenate and 2% undecylenic acid in commercial powder D[2] (in contrast to the 1% tolnaftate in commercial powder A) to bring the concentration below the minimum inhibitory concentration.

5. The mycelium remained in the first wash for 1 hr, thereby increasing contact time with the drugs. This situation gives an advantage to the less potent drug.

6. In their Table IV, where clinical isolates were used, differences were significant only one of 15 times. In Table II, seven of 15 contacts were significant. Nevertheless, the authors claimed that the results again were essentially equivalent. Furthermore, Amsel *et al.* made their conclusion on a "global basis" based on six organisms tested *in vitro*.

We compared commercial solution D (Lot 18048, May 1980) with commercial solution T[3] (Lot 7AKU2, April 1982) in a conventional agar diffusion study (3). In our studies, 5 μl of each solution was pipetted onto disks that had been placed on 23 × 23-cm plastic plates containing Sabouraud dextrose agar seeded with *Trichophyton rubrum, T. mentagrophytes,* and *Epidermophyton floccosum* (clinical isolates). Fungi were grown in Sabouraud dextrose broth on a rotary shaker at 28°. After 72 hr, the mycelia were ground into a homogeneous suspension[4]. Four milliliters of the suspension was added to 200 ml of agar and shaken, and the agar was poured and allowed to harden. Disks were removed after 1, 5, 15, and 30 min and 1 and 4 hr, and the plates were incubated at 28°. Activity (measured as zone sizes of inhibition in millimeters) against *T. mentagrophytes* was measured after 72 hr; against *T. rubrum* and *E. floccosum,* it was measured after 6 days. With all three organisms and at all times, the T solution gave much larger zones of inhibition than the D solution, even after only 1 min of contact (Table I). These differences all were highly significant.

We also compared tolnaftate (USP M.I. No. P-03411) to undecylenic acid (M.I. No. P-07413) and zinc undecylenate[5] (Lot 47E343) in minimum inhibitory concentration and agar diffusion test systems against *Microsporum canis, M. gypseum, T. rubrum, T. mentagrophytes, E. floccosum,* and *Aspergillus niger* (clinical isolates). Methods for the agar diffusion tests were as already

[1] Aftate (contains 1% tolnaftate), Plough Corp., Memphis, Tenn.
[2] Desenex (contains 10% undecylenic acid), Pharmacraft, Pennwalt Corp., Rochester, N.Y.
[3] Tinactin (contains 1% tolnaftate), Schering Corp., Kenilworth, N.J.
[4] A Brinkmann Polytron was used.
[5] City Chemical Corp., New York, N.Y.

described with several exceptions. The compounds were solubilized or suspended in acetone (0.1%). Paper disks were dipped into the acetone solution or suspensions, allowed to dry, and then placed on the agar. Disks were removed after 1, 5, and 30 min and 1, 3, and 6 hr, and zone sizes were measured after incubation for 96 hr. As in the previous experiment, tolnaftate gave larger zones of inhibition than undecylenic acid and zinc undecylenate, even after only 1 min of contact (Table II). These differences again were highly significant, with the greatest differences occurring at the early time points.

The minimum inhibitory concentration was obtained in the conventional manner (2) using Sabouraud dextrose broth (Table III). Tolnaftate again was far more active than the two undecylenates in this second model of *in vitro* testing. The minimum inhibitory concentration values for tolnaftate all were <1.0 μg/ml, while the values for the undecylenates ranged from 7.5 to >10 μg/ml after 72 hr of incubation. These differences again were highly significant.

Since our data indicated *in vitro* superiority for tolnaftate over undecylenates and since efficacy *in vivo* certainly is more meaningful, it was of interest to determine if this superiority would be seen with formulated material. Results of a double-blind study comparing commercial powder A (Lot 7A503), commercial powder C[6] (Lot 14765), and commercial talc (USP M.I. No. N-03294) against a topical *T. mentagrophytes* infection in guinea pigs are shown in Table IV. Treatment was topical (powder liberally sprinkled on infected area) twice daily for 10 days. Hair and scales were removed from the infection site and plated onto Mycosel agar every other day. In addition, animals were scored for lesion appearance every day.

Procedures for establishing, culturing, and scoring the infection were described previously (4). All animals treated with commercial powder A were negative (as measured by lack of growth of the infecting organisms) throughout the treatment period (first culture taken after 2 days of treatment), and all remained negative up to 7 days posttreatment, at which time the experiment was terminated. These results were highly significant when compared with commercial powder C. Culture results seen with commercial powder C were similar to those seen with the talc controls. During treatment, all animals treated with commercial powder C remained positive; after treatment, 33% of the cultures obtained from animals treated with commercial powder C and 29% from animals treated with talc were negative. In both groups, no negative cultures were obtained until 15 days after treatment began. In addition, the average lesion scores both during and after treatment were lower with commercial powder A than with commercial powder C, and there was no statistical difference between commercial powder C and untreated controls.

Our results with this guinea pig model are similar to those reported by other investigators using similar procedures (5). In those experiments, 1% tolnaftate powder was compared to commercial powder D to determine the activity of both substances as prophylactic agents. The results showed that 1% tolnaftate powder was far more effective than commercial powder D.

[6] Cruex (contains 10% calcium undecylenate), Pharmacraft, Pennwalt Corp., Rochester, N.Y.

In the 15 years since the guinea pig model was described, the relationship between this model and clinical effectiveness has been demonstrated for many antifungal agents. Tolnaftate (4), clotrimazole (6), and miconazole (7) all were very active in the guinea pig model, and all are presently recommended for clinical use against dermatophytes (8).

Our *in vitro* and *in vivo* data obtained following conventional testing methodology clearly indicate that tolnaftate, commercial solution T, and commercial powder A are far more active than undecylenic acid, zinc undecylenate, commercial powder C, and commercial solution D.

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Comparative *In Vitro* and *In Vivo* Antifungal Activity of Tolnaftate and Various Undecylenates: A Rebuttal

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To the Editor:

When different groups of scientists perform studies using differing techniques, one may expect somewhat different results, as evidenced by the work of Amsel *et al.* (1) and Loebenberg *et al.* (2). A rebuttal to the Loebenberg *et al.* communication (2) is clearly in order. The stress of Loebenberg *et al.* on our "unusual" methodology seems to hint that the use of a new or nonstandard method may be unacceptable. Our method (1) was reproducible by different investigators, rapid, and equivalent to older "standard" methods.

The following comments are presented in reply to the criticisms of Loebenberg *et al.* (2).

1. Their comment that minimum fungicidal concen-

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