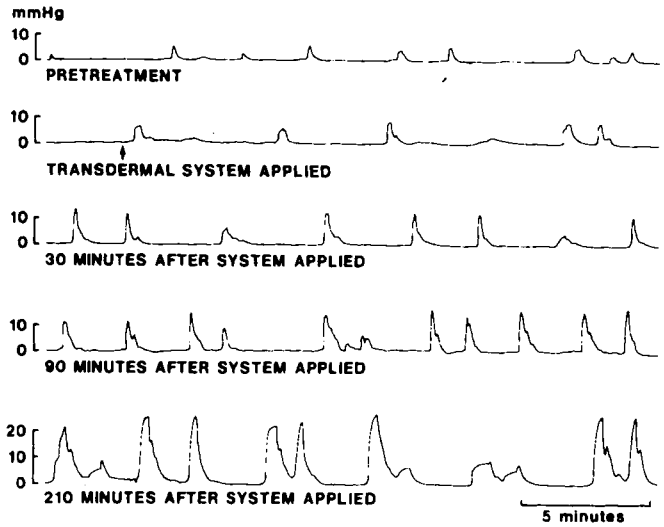

Figure 1—*Uterine motility tracing in a pregnant rhesus monkey (third trimester) treated with a transdermal delivery system containing (15S)-15-methyl prostaglandin $F_{2\alpha}$ methyl ester. Note the gradual increase in the frequency and amplitude of uterine contractions after the delivery system was applied.*

more application for the administration of therapeutic agents (4). A prostaglandin analogue has been shown to be hypotensive when administered transdermally (5). These systems may prove to be especially useful for the administration of prostaglandins for menses induction. The most commonly investigated route of administration for this indication is vaginal (6, 7). One disadvantage to the vaginal route of prostaglandin administration for menses induction is the fact that uterine bleeding is induced as a consequence of the therapy. The time of onset and the amount of uterine bleeding vary among patients. This creates a variable environment for the absorption of prostaglandin from the vagina, and can interfere with drug absorption (7). A transdermal controlled-release delivery system could alleviate these problems.

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Navel Absorption: Transdermal Bioavailability of Testosterone

Keyphrases □ Absorption—naval, transdermal, bioavailability of testosterone □ Testosterone—transdermal absorption *via* naval, bioavailability □ Bioavailability—transdermal via navel

To the Editor:

The navel (umbilicus) has for many years been widely used as the site for transdermal administration of a number of Chinese drugs, *e.g.*, *Mentha arvensis L.*[1], (1). No bioavailability data are yet available to justify the use of the navel as the site for drug administration.

Recently, a number of systemically active drugs, such as nitroglycerin and scopolamine, have been actively investigated and found to penetrate the skin tissues at a rate sufficient to achieve the therapeutic blood levels required for systemic effects (2–7).

We evaluated the transdermal bioavailability of testosterone through the skin tissues at the navel area[2] and compared the data with that obtained from the transdermal administration on the forearm area (dorsal surface) using four rhesus monkeys as the model animal. [^{14}C]-Testosterone [5 μCi (25 μg)], in 100 μL of acetone, was applied on a skin area (controlled at 0.2 cm^2 by a metal templet) for 5 d. The solvent was quickly evaporated, and the site of drug administration was then covered with a piece of plastic adhesive which remained intact throughout the study. Blood samples (2 mL) were collected in heparinized tubes at 30-min intervals for the first 6 h after administration and then every 24 h up to 9 d. Urine samples were collected daily. The systemic bioavailability and pharmacokinetic profile of [^{14}C]testosterone after topical administration on the navel and forearm skins were then compared using intravenous data as the reference.

The data generated (Fig. 1) suggest that administration *via* the navel yields a relatively faster absorption and also greater systemic bioavailability of [^{14}C]testosterone than administration *via* the forearm. Both routes of percutaneous absorption give well-defined first-order elimination kinetics, which is very much in parallel to that of intravenous administration. It is interesting to note that the systemic bioavailability of [^{14}C]testosterone by navel absorption is relatively close to the level obtained by the intravenous administration of an equivalent dose and is substantially greater than the level achieved by forearm administration. Calculated from Eq. 1, navel absorption of [^{14}C]testosterone has achieved a relative bioavailability of 79.9%, as compared with 49.9% by forearm administration.

$$\% \text{ relative bioavailability} = \frac{\left[\int_0^9 C_c dt\right]_p}{\left[\int_0^9 C_c dt\right]_i} \times 100 \qquad \text{(Eq. 1)}$$

[1] Chemical analysis indicated that it contains menthone, menthol, pinene, and methyl acetate.

[2] Navel area denotes the surface of the navel (Figure 329 in "Structure and Function in Man," 2nd ed., by S. W. Jacob and C. A. Francone, Saunders, Philadelphia, Pa., 1970).

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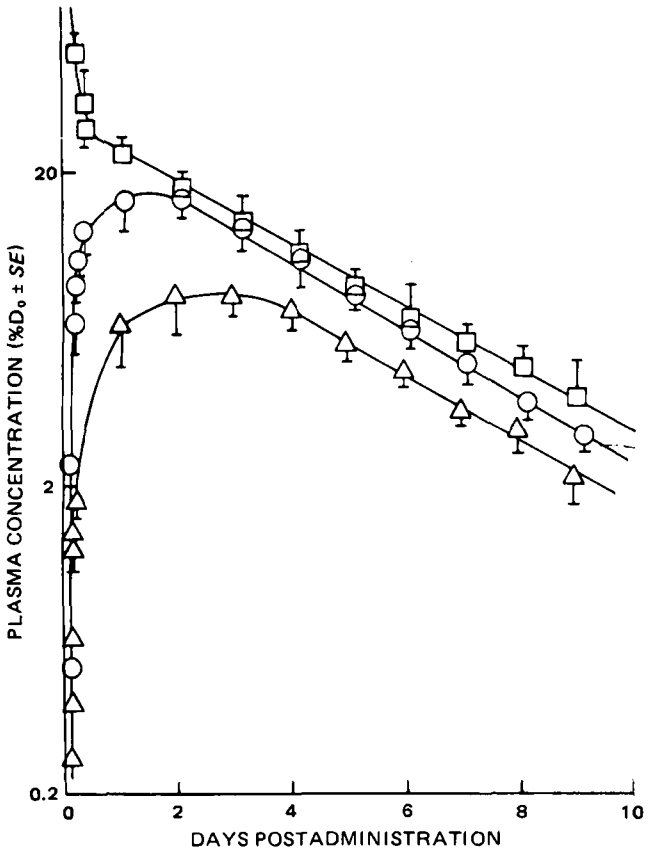

Figure 1—*Semilogarithmic relationship between the mean values (±SE) of plasma concentration (% of applied testosterone dose) and the time after various routes of administration of 5 μCi (25 μg) [^{14}C]-testosterone in four rhesus monkeys. Keys: (□) intravenous, drug in 2 mL saline solution; (○) navel, drug in 100 μL acetone; (△) forearm, drug in 100 μL acetone.*

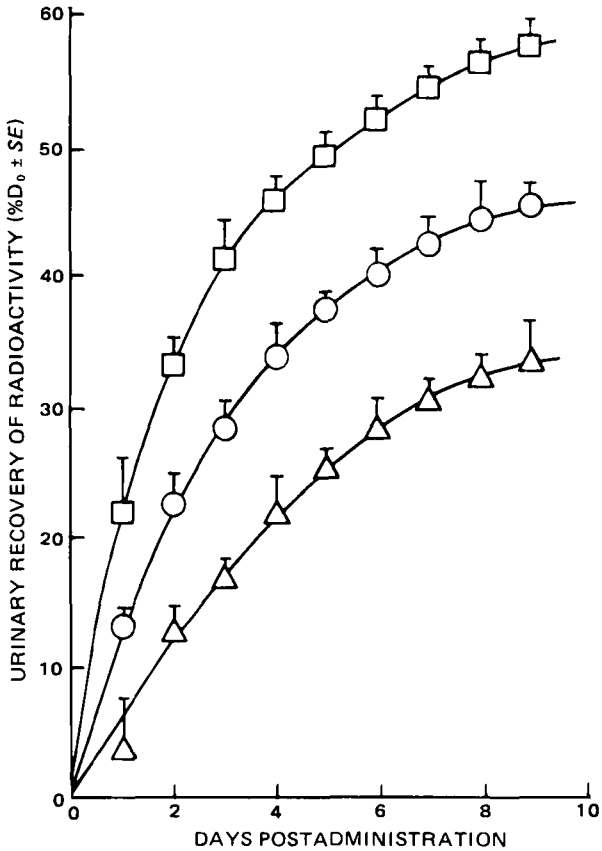

Figure 2—*Comparison of the time course of the cumulative urinary recovery of radioactive testosterone and metabolites [mean value (in % of applied dose) ±SE] following various routes of administration of 5 μCi (25 μg) [^{14}C]testosterone in four rhesus monkeys. Keys: (□) intravenous, drug in 2 mL saline solution; (○) navel, drug in 100 μL acetone; and (△) forearm, drug in 100 μL acetone.*

where $\int_0^9 C_c dt$ is the 9-day cumulative area under the plasma drug level *versus* time curve per unit dose applied (AUC/D_0) (8). And, the subscripts i and p denote, respectively, the administration of [^{14}C[testosterone *via* intravenous and percutaneous routes.

The relative bioavailability of [^{14}C]testosterone by navel and forearm absorption may also be compared using urinary recovery data. The results (Fig. 2) indicate that during a 5-day drug administration period a total of 57.3, 45.2, and 33.2% of the [^{14}C]testosterone dose administered had been recovered in urinary excretion, respectively, from intravenous, navel, and forearm administrations. Using the intravenous urinary recovery data (57.3%) as the reference for comparison, it is estimated that a relative bioavailability of 78.9% was achieved after navel administration and of 57.9% by forearm administration. The results are in good agreement with the plasma data reported (79.9 and 49.9%, respectively). Both plasma profiles and urinary recovery data demonstrate that navel absorption produces a greater transdermal bioavailability of [^{14}C]testosterone than forearm administration (79.9 and 78.9% *versus* 49.9 and 57.9%).

Additional studies were also conducted in four other rhesus monkeys with 24-h topical administration of [^{14}C]testosterone. Results (Table I) again confirmed the earlier observations that navel administration yields a higher systemic bioavailability than forearm administration (37.64 ± 3.62% *versus* 22.51 ± 1.72%).

The results lead us to conclude that a greater transdermal bioavailability of testosterone can be achieved by topical administration of drug *via* the navel route. This observation also paved the foundation for studying the feasibility of using the navel area as the site for long-term (*e.g.*, longer than 1 month) transdermal administration of testosterone *via* a controlled-release drug delivery device (9).

Table I—Comparison of the Fraction of Applied Dose Recovered in Urine after Transdermal Administration of [^{14}C]Testosterone [a]

Skin site	Monkeys	Fraction of Applied Dose Recovered in Urine, Percent[b]
Navel	#46	33.55
	#158	42.78
	#230	44.64
	#231	29.57
Mean ± *SE*		37.64 (±3.62)
Forearm	#46	18.42
	#158	21.40
	#230	23.71
	#231	26.51
Mean ± *SE*		22.51 (±1.72)

[a] 5 μCi (25 μg) of testosterone were dissolved in acetone and then applied, drop-by-drop, on either navel or forearm (0.2 cm^2). After 24 h application, the residual drug was washed off for correcting the total dose administered. [b] Calculated from: % applied dose = (Total radioactivity in the urine collected for 5 d)/(5 μCi − Residual radioactivity on the skin) × 100%.

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Variability in the Determination of Fraction Metabolized in a Triangular Metabolic Problem and Its Resolution with Stable Isotope Methodology

Keyphrases □ Cinromide—anticonvulsant, fraction metabolized □ Isotope methodology—administration of labeled metabolites □ Triangular metabolic pathway—fraction metabolized-cinromide, rhesus monkey

To the Editor:

A particular case of precursor–product relationship arises when one metabolite (metabolite II) of a parent drug is formed from two precursors, the parent drug as well as one of its proximal metabolites (metabolite I). The resolution of this metabolic problem is complicated by the fact that the fraction of parent drug metabolized directly to metabolite II can only be obtained indirectly from the following difference:

$$f_m(P \rightarrow MII, \text{direct}) = f_m(P \rightarrow MII, \text{total}) - f_m(P \rightarrow MI) \times f_m(MI \rightarrow MII) \quad \text{(Eq. 1)}$$

where f_m represents fraction metabolized, P refers to parent drug, and MI and MII refer to metabolites I and II, respectively. The classical approach to the determination of the fractions metabolized (Eq. 1) using blood data requires three separate studies: administration of parent drug, metabolite I, and metabolite II to the same subject at different times. This approach involves several assumptions of constancy of metabolite clearances among the three studies. As a result, estimates of the various fractions of Eq. 1 are subject to significant error. This type of metabolic problem arose during the elucidation of the pharmacokinetic profile of cinromide (3-bromo-*N*-ethylcinnamamide)(P), a new antiepileptic drug. This drug is metabolized by *N*-deethylation to 3-bromocinnamamide (I) and by amide hydrolysis to 3-bromocinnamic acid (II). However, I is also metabolized to II by amide hydrolysis. The resolution of this metabolic scheme prior to the efficacy evaluation of cinromide in the primate model (1, 2) was of particular significance because I was found to have anticonvulsant properties (3, 4) and II reached steady-state levels 10–20-fold higher than those of the parent drug (2). In an earlier study where the classical approach (administration of cinromide, I, and II separately) was used in a group of six rhesus monkeys, several unrealistic findings were obtained (5). The fraction of dose of cinromide metabolized directly to II ranged from 5 to 100% (mean ± *SD* = 48 ± 32%). Also, the sum of the two fractions (f_m(P → I) + f_m(P → II, direct)) ranged from 64 to 140% and was larger than 100% in three of six monkeys. This large variability was attributed to the intrasubject variability in clearances of I and II among the three studies. To test this hypothesis, a new approach using stable isotope methodology was devised. This approach involved the simultaneous administration of cinromide, and different stable isotope-labeled variants of I and II, in which case the clearances of I and II were obtained while these metabolites were formed *in situ*.

Five chaired normal male rhesus monkeys (chronically catheterized) were used in this study. Dideuterated I (I–d_2) and monodeuterated II (II–d_1) were administered along with the unlabeled parent drug (d_0). These were synthesized using the methods described for the corresponding unlabeled species (6–8). The deuterium labels were placed on the α, β ethylene carbons of I–d_2, and the β carbon of II–d_1 to minimize any possible isotope effect. Any secondary kinetic deuterium isotope effect on deethylation and amide hydrolysis or on epoxide formation and ring opening of the ethylene carbons would generally not be significant (9). Single intravenous doses of cinromide–d_0 (75 mg) and II–d_1 (80 mg) were administered consecutively to each monkey. I–d_2 (70 mg) was administered intravenously 2 h later such that the concentrations of II–d_2 would not be too far apart from those of II–d_0 and II–d_1 and could be analyzed at the same time. Solutions of cinromide–d_0, I–d_2, and II–d_1 were prepared in 60% polyethylene glycol 400 to concentrations of 25, 25, and 10 mg/mL, respectively. Blood samples (2 mL) were taken at 0.2, 0.5, 0.75, 1, 1.5, 2, 2.25, 2.5, 3, 4, 5, 6, 8, 10, 12, and 16 h after administration of II–d_1. EDTA was used as an anticoagulant in each blood sample. After the administration of cinromide–d_0, I–d_2 and II–d_1, six species were monitored in each blood sample: the three administered species as well as I–d_0 and II–d_0 formed from cinromide (d_0) and II–d_2 formed from I–d_2. Aliquots of 0.2 mL of each sample were used to analyze cinromide by HPLC (10). Aliquots of 0.5 to 0.8 mL of blood were used to analyze I and II by direct probe insertion chemical ionization mass spectrometry. Compounds I and II were separated by successive extractions at different pH. Blood samples were extracted with benzene under neutral conditions to extract I; the aqueous phases were acidified and extracted with benzene to recover II and benzene extracts containing I were washed with 0.1 M NaOH. Benzene extracts containing II were shaken with 0.1 M NaOH, then reacidified and extracted back into benzene. Chlorinated analogues of I and II were used as internal standards for

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