Table I—Maximization Grading of Cannabinoids

Cannabinoid	Sensitization Rate	Grade	Average Draize Score
I	100	V, Extreme	3.75
IV	100	V, Extreme	3.29
III	60	III, Moderate	1.69
VII	40	III, Moderate	0.38
II	30	III, Moderate	0.70
VI	0	Inactive	0
V	0	Inactive	0

sensitizers. Eugenol did not elicit cross-reactions in any animals. Since cannabinol methyl ether failed to sensitize, but did produce reactions in animals sensitized with cannabinol and cannabidiol, it appeared that a free hydroxyl group in position one was required for sensitization, but not to elicit a reaction in animals already sensitized. The failure of cannabinol methyl ether to elicit reactions in Δ^9-tetrahydrocannabinol-sensitized animals is not understood.

Although many of the biological effects of Δ^9-tetrahydrocannabinol are shared by all naturally occurring cannabinoids, the psychoactive effects are not. Desoize *et al.* (8) found that six natural cannabinoids (I, II, III, IV, VII, and cannabicyclol) suppressed phytohemagglutinin-induced DNA synthesis in normal human peripheral-blood lymphocytes, an *in vitro* model for cell-mediated immune function. In addition, the inhibitory effects of five of these six natural cannabinoids on the passive cutaneous anaphylaxis reaction in rats has been reported (9). Compound I, however, was a more potent inhibitor of passive cutaneous anaphylaxis than the other cannabinoids, and III was least active. Zimmerman *et al.* (10) reported that cannabidiol and cannabinol did not reduce hemagglutination titers to sheep red blood cells in mice at doses of 25 mg/kg, while Δ^9-tetrahydrocannabinol did.

The olivetol moiety of the molecule appeared, in the above studies, to be the portion of the molecule required for the shared activities. Olivetol was found by Desoize *et al.* (8) to inhibit phytohemagglutinin-induced lymphocyte transformation.

In this study, most cannabinoids containing the olivetol moiety were found to be skin sensitizers. Cannabinol methyl ether, which has its hydroxyl function blocked with a methyl ether, was not a sensitizer. The cross-allergenicity of these compounds is likely to be directly related to the presence of the olivetol component.

Table II—Immunological Cross-Reactivity of Cannabinoids

Sensitizing Substance	Skin Test Substance[a] I	IV	III	II	VII	VI	V	Eugenol
I	9/9	4/9	1/9	3/9	3/9	0/7	0/7	0/9
IV	2/9	9/9	0/9	3/9	1/9	4/9	4/9	0/10
III	0/6	0/6	6/10	0/6	0/10	2/6	2/6	0/6
II	0/3	0/3	0/3	3/10	0/3	NT	NT	NT

[a] Expressed as the number of animals with positive reactions to the skin test substance over the number tested.

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Absolute and Relative Bioavailability of Oral Acetaminophen Preparations

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Received February 5, 1982, from the **College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115* and the ‡*Division of Clinical Pharmacology, Tufts-New England Medical Center, Boston, MA 02111.* Accepted for publication August 11, 1982.

Abstract □ Eighteen healthy volunteers received single 650-mg doses of acetaminophen by 5-min intravenous infusion, in tablet form by mouth in the fasting state, and in elixir form orally in the fasting state in a three-way crossover study. An additional eight subjects received two 325-mg tablets from two commercial vendors in a randomized crossover fashion. Concentrations of acetaminophen in multiple plasma samples collected during the 12-hr period after each dose were determined by high-performance liquid chromatography. Following a lag time averaging 3–4 min, absorption of oral acetaminophen was first order, with apparent absorption half-life values averaging 8.4 (elixir) and 11.4 (tablet) min. The mean time-to-peak concentration was significantly longer after tablet (0.75 hr) than after elixir (0.48 hr) administration. Peak plasma concentrations and elimination half-lives were similar following both preparations. Absolute systemic availability of the elixir (87%) was significantly greater than for the tablets (79%). Two commercially available tablet formulations did not differ significantly in peak plasma concentrations, time-to-peak, or total area under the plasma concentration curve and therefore were judged to be bioequivalent.

Keyphrases □ Bioavailability—absolute and relative, oral acetaminophen preparations, determined by high-performance liquid chromatography □ Acetaminophen—absolute and relative bioavailability of oral preparations, determination by high-performance liquid chromatography □ High-performance liquid chromatography—oral acetaminophen preparations, determination of absolute and relative bioavailability

Acetaminophen (paracetamol) is used extensively as a nonprescription analgesic and antipyretic agent (1). Over 40 oral acetaminophen preparations are available commercially (2). The present study evaluated the absolute bioavailability of orally administered acetaminophen in elixir and tablet forms. Also assessed was the relative

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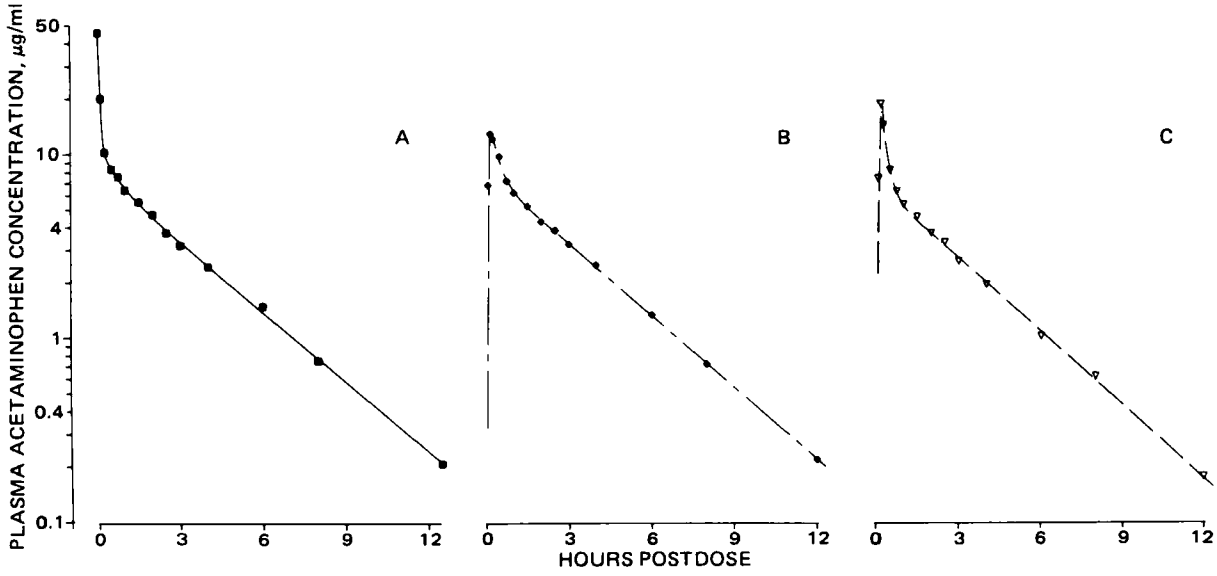

Figure 1—*Plasma acetaminophen concentrations and pharmacokinetic functions in a representative subject following 650-mg doses by three modes of administration. Key: (A) intravenous; (B) elixir; (c) tablet.*

bioavailability of two brands of oral acetaminophen tablets.

EXPERIMENTAL

Three-way Crossover Study of Acetaminophen Dosage Forms—Eighteen healthy male and female volunteers, aged 22–36 years, participated after giving informed written consent. All participants were ambulatory, were taking no other medications, and had no history of chronic disease. All subjects received a single 650-mg dose of acetaminophen on three occasions separated by at least 1 week. The sequence of the three trials was randomized. The modes of administration were:

1. Intravenous acetaminophen, administered as a sterile solution[1] infused into an antecubital vein over a period of 5 min;
2. Acetaminophen elixir[2] administered as 19.5 ml of a 33.3-mg/ml solution and followed by 20 ml of water;
3. Two 325-mg oral tablets[3] administered with 100–200 ml of water.

For the two oral dosage trials, subjects were fasted overnight prior to and for 3 hr following drug administration.

Venous blood samples were drawn from an indwelling butterfly catheter, or by separate venipuncture, and placed in heparinized tubes. Samples were collected prior to intravenous acetaminophen infusion, immediately at the end of the infusion, and at 5, 15, 30, and 45 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hr postinfusion. In the oral acetaminophen trials, a sample was drawn prior to dosage, at 5, 10, 15, 30, and 45 min, and thereafter as described for intravenous acetaminophen. Whole-blood samples were centrifuged, and the plasma was separated and stored until the time of assay.

Concentrations of acetaminophen in all plasma samples were determined by high-performance liquid chromatography (HPLC) (3). The sensitivity of this method was 0.1–0.2 μg of acetaminophen/ml of plasma. For six identical samples at concentration points ranging from 0.25–15 μg/ml, the coefficient of variation was <5%. The mean deviation between pairs of duplicate samples (n = 45) analyzed during pharmacokinetic studies was 2.4%.

Plasma acetaminophen concentrations after intravenous dosage were analyzed by iterative nonlinear least-squares techniques as described in detail previously (4, 5). Plasma levels were fitted to a linear sum of 2 or 3 exponential terms. Coefficients, corrected for the infusion period (6), and exponents from the fitted function were used to determine the total area under the plasma concentration curve from time zero to infinity ($AUC_{0\text{-}\infty}$). After oral dosage, plasma concentrations likewise were fitted to a linear sum of 2 or 3 exponential terms. Coefficients and exponents from the fitted function were used to determine the apparent lag time prior to the start of absorption, the first-order absorption half-life, and the elimination half-life (7). The plasma concentration AUC from time zero until the final detectible acetaminophen concentration was determined by the trapezoidal method. To this value was added the residual area extrapolated to infinity, calculated as the final plasma concentration divided by the terminal exponent. The sum of these two areas represent the total AUC (7, 8).

Absolute bioavailability of both oral acetaminophen preparations for each subject was determined as the AUC following oral administration divided by the AUC following intravenous dosage to the same subject. Statistical methods included Student's t test and ANOVA.

Two-way Crossover Study of Two Acetaminophen Tablets—The

Table I—Pharmacokinetics of Intravenous Acetaminophen

Subject	Age	Sex	Volume of Distribution, liters/kg	β Half-life, hr	Clearance, mg/min/kg	$AUC_{0\text{-}\infty}$, μg/ml · hr
1	25	F	1.27	2.65	5.55	41.0
2	25	F	0.76	2.70	3.27	50.3
3	33	F	0.90	2.15	4.85	44.7
4	25	F	0.66	2.78	2.73	72.8
5	23	F	1.07	2.94	4.20	47.3
6	32	F	0.88	2.32	4.37	48.8
7	29	F	0.95	3.06	3.59	53.2
8	26	F	0.99	2.83	4.06	49.8
9	22	M	0.97	2.64	4.24	31.2
10	33	M	1.02	3.02	3.92	42.0
11	24	M	1.36	2.21	7.11	21.6
12	24	M	1.13	1.88	6.94	22.2
13	39	M	0.99	2.55	4.48	28.0
14	39	M	1.34	2.90	5.53	22.6
15	30	M	1.09	2.54	4.93	32.2
16	26	M	1.00	2.61	4.45	35.7
17	22	M	0.91	2.39	4.36	34.2
18	25	M	1.52	3.19	5.50	31.0
Mean	27.9		1.05	2.63	4.67	39.37
± SE	1.3		0.05	0.08	0.27	3.12

Table II—Pharmacokinetic Parameters for Two Oral Preparations of Acetaminophen [a]

Parameter	Mean ± SE Values		Student's t test
	Elixir	Tablet	
Peak plasma concentration, μg/ml	12.41 ± 1.22	11.99 ± 1.02	0.34
Time-to-peak concentration, hr	0.48 ± 0.06	0.76 ± 0.12	2.54[b]
Lag time, hr	0.06 ± 0.01	0.07 ± 0.01	0.58
Absorption half-life, hr	0.14 ± 0.02	0.19 ± 0.04	1.03
Elimination half-life, hr	2.74 ± 0.13	2.55 ± 0.14	1.78
Systemic availability[c]	0.87 ± 0.02	0.79 ± 0.02	4.47[d]

[a] In the three-way crossover study. [b] Significance level = $p < 0.025$. [c] Fraction of intravenous. [d] Significance level = $p < 0.001$.

[1] Thirteen milliliters of a 50-mg/ml solution [propylene glycol–ethyl alcohol–5% dextrose (40:10:50, v/v)] diluted to 50 ml with 5% dextrose.
[2] McNeil, Fort Washington, Pa.
[3] Parke-Davis, Ann Arbor, Mich.

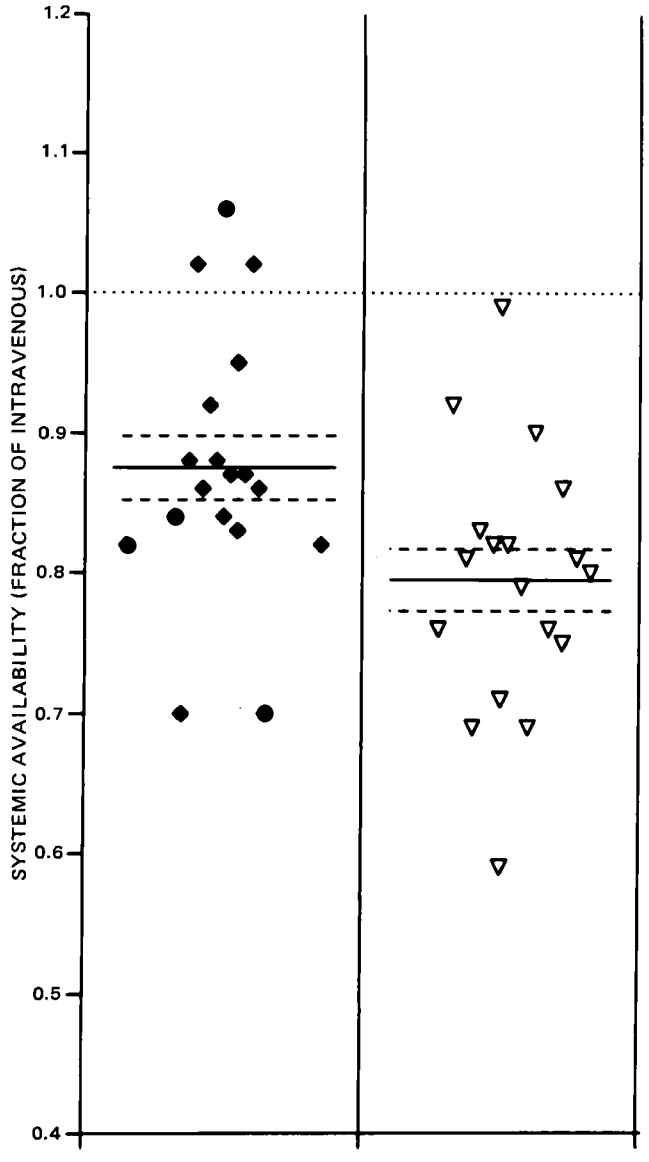

Figure 2—*Absolute bioavailability of oral acetaminophen in the elixir (◆) and tablet (▽) preparations. Horizontal lines represent means (—), standard errors (- - -), and 100% of the intravenous bioavailability (·····).*

relative bioavailability of two oral acetaminophen tablet preparations was evaluated in a two-way randomized crossover study involving seven male and one female volunteer. All subjects received a single 650-mg dose (two 325-mg tablets) of oral acetaminophen on two occasions separated by at least one week, using two commercially available[4] products (A and B).

Plasma samples were obtained as described above following both oral acetaminophen trials. Acetaminophen concentrations in all samples were determined by HPLC (3). Pharmacokinetic and statistical analyses were as described above. Differences between the two oral preparations were evaluated by Students's t test.

RESULTS

Comparison of Intravenous, Elixir, and Tablet Dosage Forms—Following intravenous acetaminophen administration, disappearance of drug from plasma was described by a linear sum of exponential terms (Fig. 1 and Table I). Iterative solutions were possible for 15 subjects following acetaminophen elixir administration and for 11 subjects following acetaminophen dosing by tablet (Fig. 1).

[4] Brand A was Tylenol tablets, lot HP2588, McNeil Consumer Products Co., Fort Washington, Pa.; Brand B was Tapar tablets, lot 2C246, Parke-Davis, Ann Arbor, Mich.

Table III—Pharmacokinetic Parameters for Two Tablet Formulations of Acetaminophen

Parameter	Mean ± *SE* Values Brand A	Brand B	Student's Paired t
Peak plasma concentration, μg/ml	8.56 ± 1.22	8.89 ± 0.78	0.221
Time-to-peak concentration, hr	1.06 ± 0.27	0.78 ± 0.10	0.986
Elimination half-life, hr	2.62 ± 0.10	2.43 ± 0.11	2.30[a]
Total $AUC_{0\to\infty}$, μg/ml × hr	26.82 ± 1.44	27.37 ± 0.94	0.468

[a] Significance level $0.05 < p < 0.10$.

Peak plasma concentrations following the elixir preparation administration were slightly higher than that for tablets, but the difference was not significant (Table II). The time-to-peak concentration averaged 0.48 hr after dosage with elixir *versus* 0.75 hr with the tablet ($p < 0.025$). The two preparations did not differ significantly in apparent half-life of absorption or in lag time prior to the start of absorption.

Absolute systemic availability of both oral preparations was significantly less than 100% complete (Table II, Fig. 2). Absolute availability of the elixir (87%) was significantly greater than that for tablets (79%). The elimination half-lives were similar following both preparations (Table II).

Comparison of Two Acetaminophen Tablet Formulations—The kinetics of acetaminophen absorption following dosing with brand B were very close to that reported in the three-way crossover study. Peak plasma concentration averaged 8.9 μg/ml and was reached an average of 0.78 hr after dosing. These values were similar to those observed for brand A (8.6 μg/ml and 1.1 hr after dosing, respectively), and the differences did not approach significance. The total AUC was nearly identical for both preparations (Fig. 3, Table III). The elimination half-life following brand B administration (2.43 hr) was shorter than with brand A (2.62 hr) although the magnitude of the mean difference was only 8%.

DISCUSSION

The results of the three-way crossover study concur with reports of other investigators (9) and indicate that absorption of acetaminophen from both tablet and elixir preparations is relatively rapid, with peak plasma concentrations generally attained within 1 hr postdose. The time-to-peak concentration was significantly shorter with the elixir than with the tablet, but differences in other absorption kinetic parameters did not reach statistical significance. Generally, drug absorption from an elixir preparation will be somewhat more rapid than from the same dose administered as a tablet. Absorption of acetaminophen from oral tablet preparations can be dissolution rate limited (10), probably explaining why the time-to-peak concentration was earlier following the elixir than with the tablet.

Neither preparation showed complete (100%) systemic availability;

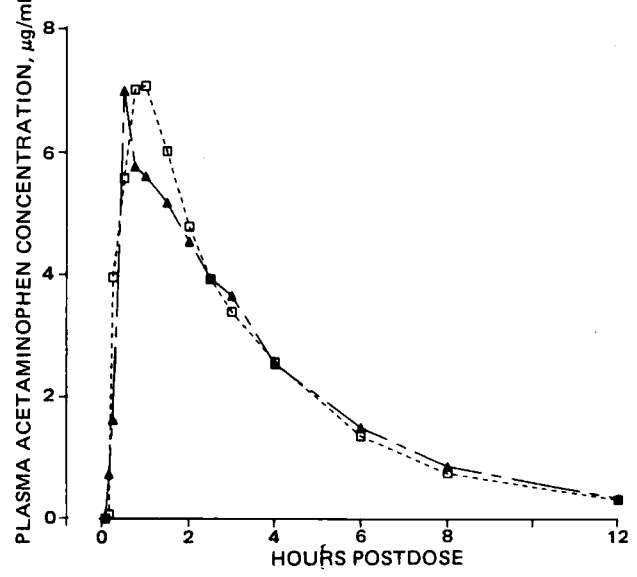

Figure 3—*Plasma concentrations following 650-mg doses of two brands of acetaminophen. Each point is the mean for all subjects at that time point. Key: (▲) Brand A; (□) Brand B.*

the elixir had significantly greater bioavailability than did the tablet. The incomplete systemic availability of oral acetaminophen could be explained either by incomplete absorption or presystemic biotransformation, (*e.g.*, first-pass hepatic extraction or metabolism in the epithelium and/or lumen of the GI tract), or by a combination of these two factors (11). Differentiation between these two possible mechanisms could be achieved through an examination of route-dependent differences in the pattern of drug metabolism, as described by Harris and Riegelman (12). In a previous study of systemic availability (13), Rawlins *et al.* reported the bioavailability of acetaminophen tablets in a 500-mg dose to be only 63% compared with 79% in our study of a 650-mg dose. Bioavailability increased to 89 and 87% following 1,000- and 2,000-mg doses, respectively (13). The discrepancy in results of the two studies may be due to saturation of presystemic biotransformation at doses >500 mg.

The comparative bioavailability studies of two widely used acetaminophen tablets suggest that they have essentially similar systemic availability and therefore should be therapeutically equivalent.

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Simultaneous Determinations of Cefsulodin and Cefotiam in Serum and Bone Marrow Blood by High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic method is described for the simultaneous determinations of cefsulodin and cefotiam in serum and bone marrow blood samples. After extraction with acetonitrile, the cephalosporins were applied to a reverse-phase column with an internal standard, cefazolin; the mobile phase was a mixture of 0.005 *M* tetrabutylammonium phosphate and methanol (35:65, v/v). The method yielded satisfactory resolutions for these agents, and the results were compared with those obtained using the microbiological method. The statistical analysis of the relationship between the methods gave a good correlation for all of these agents and samples. The concentrations of cefsulodin and cefotiam, concurrently administered by the intravenous route to patients subjected to artificial total joint prosthesis, in serum and bone marrow blood collected at 0.5 and 1 hr postinjection were almost equivalent.

Keyphrases □ Cefsulodin—simultaneous determination with cefotiam in serum and bone marrow blood, high-performance liquid chromatography □ Cefotiam—simultaneous determination with cefsulodin in serum and bone marrow blood, high-performance liquid chromatography □ High-performance liquid chromatography—simultaneous determination of cefsulodin and cefotiam in serum and bone marrow blood

Cefsulodin, sodium 4-carbamoyl-1-[(6*R*,7*R*)-2-carboxy-8-oxo-7- [(2*R*) -2-phenyl-2-sulfoacetamido] -5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methylpyridium hydroxide, a potent cephalosporin derivative, is superior to sulbenicillin and carbenicillin and comparable to gentamicin in activity against *Pseudomonas aeruginosa*. The drug is stable to *P. aeruginosa*-specific cephalosporinase (1–3). Cefotiam, (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)-acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid dihydrochloride, also shows broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (4). Combined administration of these agents is frequently used to treat systemic infections in which a broader anti-infective spectrum is needed. A simple, specific high-performance liquid chromatographic (HPLC) method was developed which determines both cefsulodin and cefotiam in biological fluids. A comparison is made with the previously used microbiological method.

EXPERIMENTAL

Materials—Cefsulodin[1] and cefotiam[2] were used as received. Cefazolin[3], employed as an internal standard, was used as received. HPLC-quality methanol[4] and acetonitrile[5] were used. Tetrabutylammonium

[1] Tilmapor, Ciba-Geigy, Basle, Switzerland.
[2] Halospor, Ciba-Geigy, Basle, Switzerland.
[3] Cefamezin, Fujisawa Pharmaceutical Co., Osaka, Japan.
[4] Wako Chemical Co., Osaka, Japan.
[5] Tokyo Kasei Chemical Co., Tokyo, Japan.

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