Percutaneous Absorption of Flufenamic Acid in Rabbits: Effect of Dimethyl Sulfoxide and Various Nonionic Surface-Active Agents

CHIAW-CHI HWANG and AUGUST G. DANTI ˣ

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Abstract □ Eight nonionic surface-active agents were each incorporated at a concentration of 10% into a white petrolatum ointment base containing 10% flufenamic acid with or without dimethyl sulfoxide. Percutaneous absorption was studied by determining the plasma concentration of flufenamic acid in New Zealand White rabbits at regular intervals for 8 hr following application of the ointment. The percutaneous absorption of flufenamic acid was significantly increased when sorbitan trioleate, polyoxyl 8 stearate, or polyoxyethylene 2 oleyl ether were added to the ointment containing flufenamic acid and white petrolatum. The percutaneous absorption of flufenamic acid was increased significantly when sorbitan monopalmitate, sorbitan trioleate, polyoxyl 8 stearate, polyoxyethylene 20 cetyl ether, or polyoxyethylene 2 oleyl ether were added to the ointment containing dimethyl sulfoxide, flufenamic acid, and white petrolatum.

Keyphrases □ Flufenamic acid—percutaneous absorption, effect of nonionic surfactants and/or dimethyl sulfoxide □ Dimethyl sulfoxide—effect on the percutaneous absorption of flufenamic acid, synergism with nonionic surfactants □ Surfactants, nonionic—effect on the percutaneous absorption of flufenamic acid, synergism with dimethyl sulfoxide □ Absorption, percutaneous—of flufenamic acid, effect of dimethyl sulfoxide and/or nonionic surfactants

The anti-inflammatory and analgesic actions of flufenamic acid, *N*-(α,α,α-trifluoro-*m*-tolyl)-anthranilic acid, have been reported previously (1–4). The side effects shown on oral administration of flufenamic acid are predominantly GI disorders such as diarrhea, nausea, and vomiting. Cutaneous application of flufenamic acid for rheumatic disorders could have numerous advantages. A highly potent preparation could be used without causing the aforementioned GI disturbances. The active ingredients diffuse directly through the skin at the application site, and the concentration of the active ingredients in the subcutis and the superficial musculature are considerably higher with a longer duration of action than after oral administration. Percutaneous absorption of flufenamic acid and the resulting anti-inflammatory activity has been shown using animal and human models (5–10).

The use of dimethyl sulfoxide as a penetrant carrier has been suggested (11). Stoughton *et al.* (12, 13) reported that dimethyl sulfoxide increased the rate of penetration of naphazoline hydrochloride, hexopyrronium bromide, and fluocinolone acetonide through the human skin. Dimethyl sulfoxide also has been found to accelerate the penetration of water (14), hydrocortisone, and testosterone (15) through the skin *in vivo*.

Surfactants are one of the most important groups of adjuvants in pharmaceutical preparations. For topically applied preparations, surfactant-induced dissolution or emulsification of active ingredients and changes in ointment viscosity may modify the absorption process. Higuchi (16) suggested that the surfactants generally possess a particular affinity for membranous structure. As a result of this affinity, a nonionic surfactant could possibly emulsify the sebum, enhance the thermodynamic activity of drugs, or change the diffusion constant and activity coefficient of drugs, all of which would permit easier penetration of the drug into the cells.

Shen *et al.* (17) studied the effects of 15 nonionic surfactants on the percutaneous absorption of salicylic acid in white petrolatum containing dimethyl sulfoxide. They found that the plasma salicylate levels in rabbits were increased significantly when sorbitan trioleate, sorbitan monopalmitate, poloxamer 231, poloxamer 182, polyoxyethylene 4 lauryl ether, polyoxyethylene 2 oleyl ether, or polyoxyl 8 stearate was added to the ointment. As a continuation of this work, the effect of selected nonionic surfactants on the percutaneous absorption of flufenamic acid in the presence of dimethyl sulfoxide was studied in rabbits.

EXPERIMENTAL

Materials—The following ointments were used: 10% flufenamic acid[1] in white petrolatum[2], 10% flufenamic acid plus 5% dimethyl sulfoxide[3] in white petrolatum, 10% flufenamic acid plus 10% surfactant in white petrolatum, and 10% flufenamic acid plus 10% surfactant plus 5% dimethyl sulfoxide in white petrolatum. The nonionic surfactants selected [with hydrophilic–lipophilic balance (HLB) values in brackets] were sorbitan monopalmitate[4] [6.7], sorbitan trioleate[5] [1.8], polyoxyl 8 stearate[6] [11.1], polyoxyl 40 stearate[7] [16.9], polyoxyethylene 20 cetyl ether[8] [15.7], polyoxyethylene 2 oleyl ether[9] [4.9], poloxamer 184[10] [15], and poloxamer 231[11] [2]. The absorption of topically applied flufenamic acid was compared for white petrolatum preparations containing the acid (with or without dimethyl sulfoxide) *versus* the acid (with or without dimethyl sulfoxide) plus a surfactant.

Ointment Preparation—Flufenamic acid, in a fine powder form, was dried at 50° in a heated vacuum desiccator for at least 48 hr before use. The prepared ointments contained 10% (w/w) flufenamic acid and 10% (w/w) surfactant with or without 5% dimethyl sulfoxide. Each ingredient was weighed on an analytical balance[12] and incorporated into the white petrolatum by the fusion method.

Test Animals—Each ointment was applied to the skin of a New Zealand white rabbit weighing 3.3–4.3 kg. Each rabbit was used four times and received the same group of surfactants in each test. Not more than three rabbits were utilized during any one experimental day, due to space and time limitations. The rabbits receiving ointments containing dimethyl sulfoxide for the first test run received the ointment without dimethyl sulfoxide for the second run and *vice versa*, with a 7-day rest period before reapplication. The animals were offered food[13] and water *ad libitum* and were housed individually in an animal room maintained

[1] Reagent grade, lot 0715KE; Aldrich Chemical Co.
[2] Reagent grade, lot 5M05; Matheson Coleman & Bell.
[3] Reagent grade, lot 755838; Fisher Scientific Co.
[4] Span 40; Atlas.
[5] Span 85; Atlas.
[6] Myrj 45; Atlas.
[7] Myrj 52; Atlas.
[8] Brij 58; Atlas.
[9] Brij 93; ICI.
[10] Pluronic L64; Wyandotte.
[11] Pluronic L81; Wyandotte.
[12] Model EA-1; Torsion Balance Co., Clifton, N.J.
[13] Purina rabbit chow; Ralston-Purina Co., St. Louis, Mo.

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Table I—AUC_{0-8} for Plasma Concentration(s) of Flufenamic Acid *Versus* Time for Formulations With and Without Dimethyl Sulfoxide [a]

Surfactant	I [b]	II [c]
None	7.400 ± 1.332	21.784 ± 4.369
Sorbitan monopalmitate	5.421 ± 0.730	38.721 ± 1.016
Sorbitan trioleate	38.511 ± 12.976	123.194 ± 1.096
Polyoxyl 8 stearate	16.532 ± 4.131	64.462 ± 3.662
Polyoxyl 40 stearate	7.456 ± 0.373	11.751 ± 0.254
Polyoxyethylene 20 cetyl ether	1.789 ± 0.266	37.753 ± 5.044
Polyoxyethylene 2 oleyl ether	23.066 ± 8.460	109.314 ± 19.114
Poloxamer 184	2.610 ± 0.610	10.023 ± 0.608
Poloxamer 231	8.455 ± 2.749	26.384 ± 6.538

[a] Percutaneous absorption in rabbits; average of three determinations. [b] Flufenamic acid, surfactant, and white petrolatum. [c] Flufenamic acid, surfactant, dimethyl sulfoxide, and white petrolatum.

at a temperature of 25°. Fifteen to eighteen hours prior to the application of the ointment, the hair was removed from the back of the rabbit (8 × 12-cm^2 area) with an animal clipper[14] and depilatory cream[15]. The skin was examined under low-power magnification for damage resulting from the shaving procedure, and the animal was not used if the skin barrier was disrupted.

Application of Ointment—The rabbits were immobilized in a rack (stock) during treatment to prevent them from ingesting the ointment after application. The selected ointment was uniformly spread over the shaved back in a variety of doses (1 g ointment/kg). The ointment remained in contact with the skin for 8 hr, during which time the rabbit did not receive food or water.

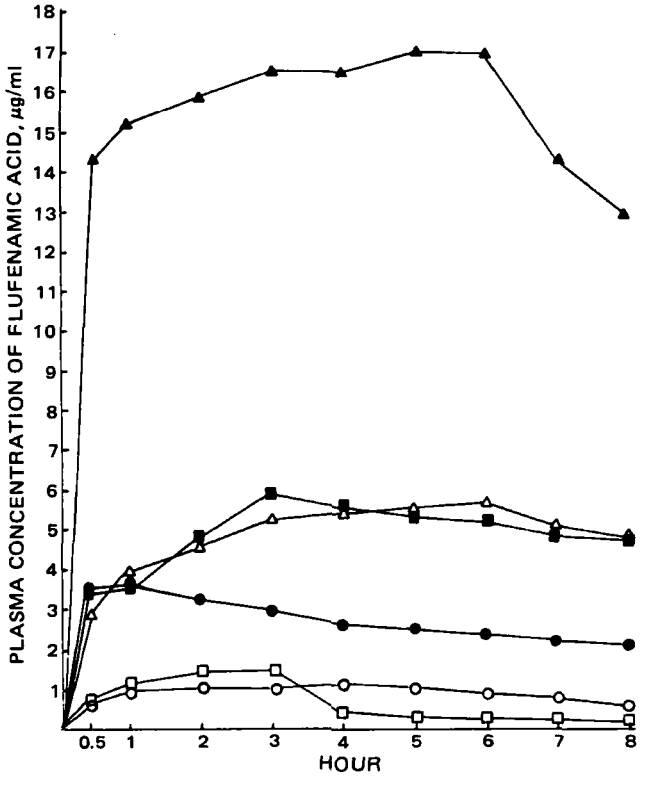

Figure 1—*Effect of sorbitan surfactants on the percutaneous absorption of flufenamic acid with or without dimethyl sulfoxide. Flufenamic acid in white petrolatum (○) with (□) sorbitan monopalmitate, (△) sorbitan trioleate, (●) dimethyl sulfoxide, (■) sorbitan monopalmitate plus dimethyl sulfoxide, and (▲) sorbitan trioleate plus dimethyl sulfoxide.*

[14] Oster Co.
[15] Neet; Whitehall Laboratory Inc., New York, N.Y.

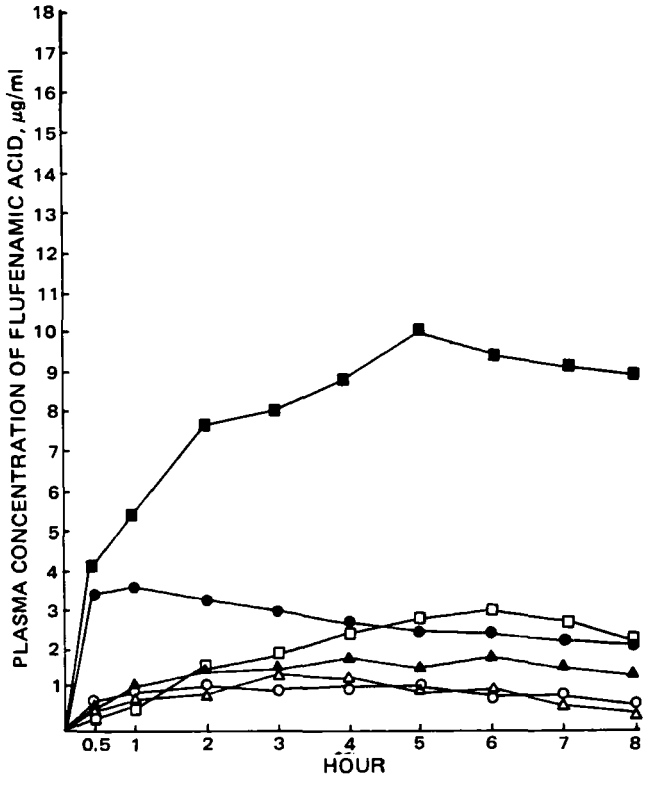

Figure 2—*Effect of polyoxyethylene ester surfactants on the percutaneous absorption of flufenamic acid with or without dimethyl sulfoxide. Flufenamic acid in white petrolatum (○) with (□) polyoxyl 8 stearate, (△) polyoxyl 40 stearate, (●) dimethyl sulfoxide, (■) polyoxyl 8 stearate plus dimethyl sulfoxide, and (▲) polyoxyl 40 stearate plus dimethyl sulfoxide.*

Procedure of Sample Collection—Blood samples were collected and analyzed for flufenamic acid. One-half milliliter of blood was withdrawn from the marginal ear vein of the rabbit prior to application of the ointment, at 0.5 hr after ointment application, and at hourly intervals for 8 hr after application. The blood was collected with a sterile 26-gauge, 0.9-cm needle[16] in a 1-ml disposable tuberculin syringe containing 0.05 ml of sodium heparin[17]. This blood–heparin mixture was placed in a 15-ml glass-stoppered centrifuge tube containing 0.5 ml of 0.2 *M* acetate buffer and 6 ml of ethyl acetate.

The concentration of flufenamic acid was analyzed using the spectrofluorometric[18] method described by Hattori *et al.* (18). Concentrations were determined using a standard curve obtained by analysis of heparinized (0.05 ml) blood samples with added known amounts of flufenamic acid. The plasma sample obtained from each rabbit prior to the drug application was used as a blank to determine the background fluorescence for that animal.

Statistical Analysis of Data—Three replications were made of each determination. A *t* test with four degrees of freedom at the 95% significance level was used to test the null hypothesis.

RESULTS AND DISCUSSION

The altered percutaneous absorption patterns of flufenamic acid obtained on the addition of surfactants to flufenamic acid, with or without dimethyl sulfoxide, in white petrolatum ointments are shown in Figs. 1–4. The areas under the curves (AUC) for the plasma concentration of flufenamic acid *versus* time of the different treatments were evaluated from 0 to 8 hr postdose (AUC_{0-8}) using the trapezoidal rule (Table I). The *t* test results for the comparison of the AUC_{0-8} between flufenamic acid (with or without dimethyl sulfoxide) plus a surfactant and flufenamic acid (with or without dimethyl sulfoxide) are shown in Table II.

Statistical analyses of the results of this study indicated that some of the surfactants functioned as penetrant carriers, enhancing the percu-

[16] Lot No. 327322, Sherwood Medical Industries Inc.
[17] 10,000 U/ml, lot No. 4CS09A; Eli Lilly & Co.
[18] Spectrophotofluorometer, Serial No. D223-62155; American Instrument Co., Silver Spring, Md.

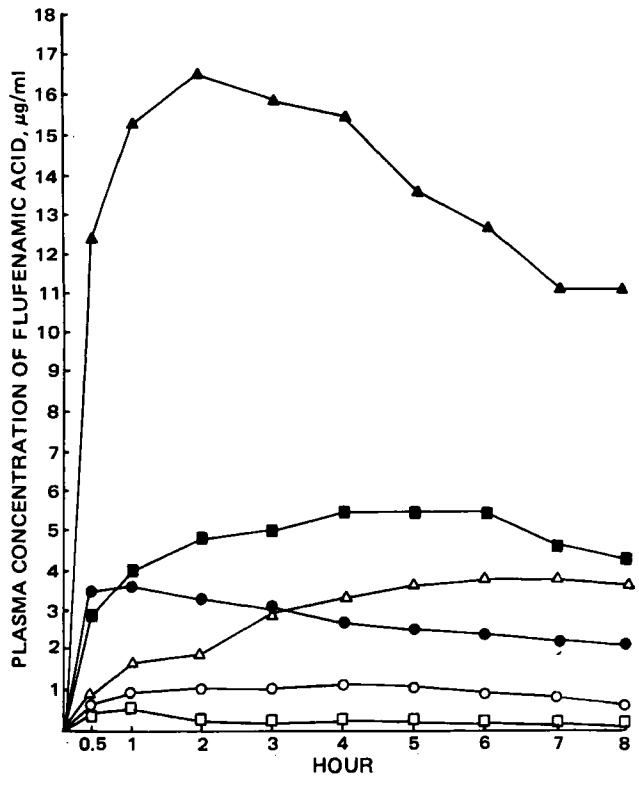

Figure 3—*Effect of polyoxyethylene surfactants on the percutaneous absorption of flufenamic acid with or without dimethyl sulfoxide. Flufenamic acid in white petrolatum (○) with (□) polyoxyethylene 20 cetyl ether, (△) polyoxyethylene 2 oleyl ether, (■) polyoxyethylene 20 cetyl ether plus dimethyl sulfoxide, (▲) polyoxyethylene 2 oleyl ether plus dimethyl sulfoxide, and (●) dimethyl sulfoxide.*

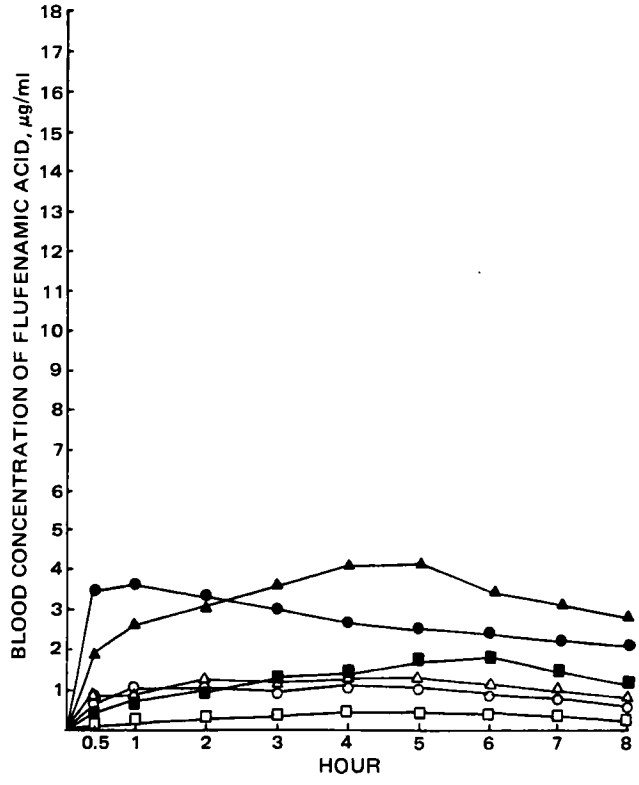

Figure 4—*Effect of poloxamer surfactants on the percutaneous absorption of flufenamic acid with or without dimethyl sulfoxide. Flufenamic acid in white petrolatum (○) with (□) poloxamer 184, (△) poloxamer 231, (●) dimethyl sulfoxide, (■) poloxamer 184 plus dimethyl sulfoxide, and (▲) poloxamer 231 plus dimethyl sulfoxide.*

taneous absorption of flufenamic acid. When these surfactants were added to an ointment containing flufenamic acid and dimethyl sulfoxide, percutaneous absorption of flufenamic acid was significantly increased throughout the 8-hr experimental period. The data suggest that this was a synergistic effect between the surfactant and dimethyl sulfoxide.

The percutaneous absorption of flufenamic acid was increased when dimethyl sulfoxide was added to the ointment. Since dimethyl sulfoxide (11) and flufenamic acid have been used alone in the treatment of musculoskeletal disorders, it was deemed worthwhile to conduct a further pharmacological study using concomitant cutaneous application of these two substances.

Dimethyl sulfoxide exhibited an unusual concentration dependence. Some investigators have pointed out that at least 60% dimethyl sulfoxide was required for a measurable permeability change in the skin (19). In this study, the percutaneous absorption of flufenamic acid was increased significantly by the addition of 5% dimethyl sulfoxide. The lower percutaneous absorption of flufenamic acid with certain surfactants may be due to the lowering of the thermodynamic activity of flufenamic acid by complexation or by other interactions with the skin (20) or by miceller trapping of the active ingredient (21).

Both the mechanism by which the percutaneous absorption of flufenamic acid is increased on addition of nonionic surfactants in the presence of dimethyl sulfoxide and the mechanism of action of dimethyl sulfoxide itself are unknown. However, it can be concluded that ultrastructural modifications of the stratum corneum caused by dimethyl sulfoxide and/or the surfactants, associated with altered skin permeability (22) do occur. Higuchi (16) suggested that the activity coefficient plays a major role in percutaneous absorption. Flufenamic acid may be held firmly by the white petrolatum, which exhibits a low activity coefficient. When dimethyl sulfoxide and the surfactants are added to the flufenamic acid in white petrolatum, the release rate of flufenamic acid could be increased by forming high activity coefficient complexes such as dimethyl sulfoxide–drug, surfactant–drug, or dimethyl sulfoxide–surfactant–drug.

Table II—*t* Test Comparisons of AUC_{0-8} for Plasma Concentrations of Flufenamic Acid for Formulations With *Versus* Without Surfactant [a]

Surfactant	I[b]	II[c]
Sorbitan monopalmitate	2.256[d]	6.543[e]
Sorbitan trioleate	4.131[e]	39.009[e]
Polyoxyl 8 stearate	3.644[e]	12.970[e]
Polyoxyl 40 stearate	0.070	3.972[d]
Polyoxyethylene 20 cetyl ether	7.155[d]	4.145[e]
Polyoxyethylene 2 oleyl ether	3.169[e]	7.732[e]
Poloxamer 184	5.663[d]	4.620[d]
Poloxamer 231	0.598	1.013

[a] $t = 5.457$ for the comparison of flufenamic acid in white petrolatum with *versus* without dimethyl sulfoxide. [b] Flufenamic acid in white petrolatum with *versus* without surfactant. [c] Flufenamic acid plus dimethyl sulfoxide in white petrolatum with *versus* without surfactant. [d] Statistically significantly less, $p < 0.05$. [e] Statistically significantly greater, $p < 0.05$.

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Steady-State Determination of the Contribution of Lung Metabolism to the Total Body Clearance of Drugs: Application to Carbamazepine

PETER J. WEDLUND, SHIH-LING CHANG, and RENÉ H. LEVY[x]

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Abstract □ A steady-state approach is proposed to examine the contribution that the lung makes to the total body elimination of medium- to high-clearance drugs. Carbamazepine, a potential candidate of pulmonary metabolism, was investigated by infusion into the femoral vein in seven unrestrained Sprague–Dawley rats (250–300 g). Blood samples (0.45 ml), taken simultaneously from the jugular vein and carotid artery in each rat during the infusion (2–5 days), were assayed in duplicate for carbamazepine by GLC/CI/MS. Venous blood concentrations were used to calculate the total body clearance of carbamazepine, 440 ± 38 ml/hr (mean ± *SEM*), and the difference between simultaneous venous and arterial blood concentrations were used to calculate the extraction ratio of carbamazepine by the lung. The mean extraction ratio of 0.0058 (n = 28) suggests that the lung only contributes ~5% to the total body clearance of carbamazepine. It is proposed that this technique could be useful in examining the importance of the lung in the total body clearance of other drugs, and that it has several advantages over some currently used techniques.

Keyphrases □ Carbamazepine—elimination *via* pulmonary metabolism in the rat, steady-state determination □ Metabolism, pulmonary—of carbamazepine in the rat, steady-state determination of drug elimination *via* the lungs □ Drug clearance—contribution to lung metabolism, steady-state determination using carbamazepine in the rat

Numerous articles and reviews have appeared over the last 10 years establishing the xenobiotic-metabolizing capability of *in vitro* lung preparations (1–5). However, the extrapolation of *in vitro* data on pulmonary metabolism to drug elimination by the lungs *in vivo* is fraught with difficulties and limitations (6–8). Several approaches are available for quantitation of lung metabolism *in vivo*, including isolated lung perfusion (6–9), ratios of area under the curve following venous and arterial bolus doses (10), and measurement of the extraction of drug across the lung at steady state (11). While each approach has advantages and disadvantages, measurement of drug extraction across the lung following achievement of steady-state drug levels constitutes a reliable and convenient method of delineating the contribution of the lung to the total body clearance of drugs. In the present study, the steady-state approach was used in rats to investigate the possible contribution of the lung to the total body clearance of the antiepileptic drug,

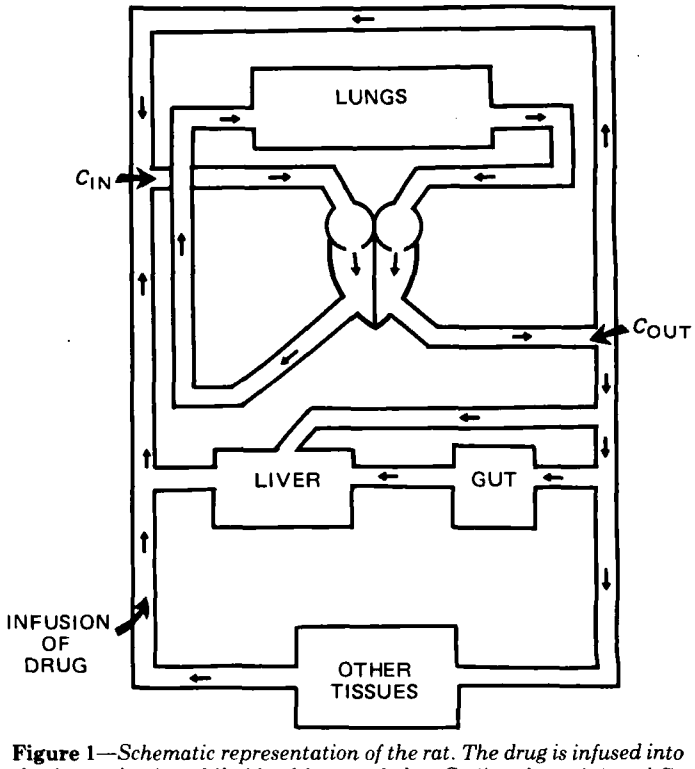

Figure 1—*Schematic representation of the rat. The drug is infused into the femoral vein, while blood is sampled at* C_i *(jugular vein) and* C_o *(carotid artery).* $CL_L = Q_B \times ER$ *[lung clearance = lung blood flow ×* $(C_i - C_o)/C_i$*].*

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