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Triprolidine Radioimmunoassay: Disposition in Animals and Humans

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Abstract □ A hapten derivative of triprolidine, bearing an acrylic acid side chain *ortho* to the pyridine ring nitrogen atom, was synthesized and coupled to bovine serum albumin. Immunization of New Zealand White rabbits with the resulting drug-protein conjugate resulted in the production of antisera capable of binding a radioiodinated tyramine conjugate of the triprolidine hapten derivative at high antiserum dilutions (1:70,000–1:150,000). These antisera were used to develop a radioimmunoassay (RIA) for triprolidine in human plasma with a sensitivity limit of 0.1 ng/mL (0.01 ng of actual mass). The known hydroxymethyl and carboxyl metabolites of triprolidine cross-reacted weakly (<2 and <0.05%, respectively) with this antiserum. The RIA could be used for the direct analysis of triprolidine in human and rabbit plasma, but not for rat or dog plasma, presumably due to the presence of other interfering substances (possibly metabolites). The validity of the RIA procedure in human plasma was demonstrated by comparative analysis of a number of samples by quantitative TLC (r = 0.985, slope = 1.076). The assay was employed to describe the pharmacokinetics of triprolidine in the rabbit ($t_{1/2,\beta}$ = 1.7 h). The assay had adequate sensitivity to detect low circulating drug concentrations in humans after therapeutic oral doses and also substantiated previous disposition experiments with triprolidine in humans ($t_{1/2,\beta}$ = 2.27 h). TLC analysis demonstrated that the absolute oral bioavailability of triprolidine (1-mg/kg dose) in the dog was low (4%). A comparison of triprolidine pharmacokinetic parameters in dogs, rabbits, rats, and humans revealed considerable similarity in elimination characteristics in these species.

Keyphrases □ Triprolidine—bovine serum albumin conjugate, pharmacokinetics, animal-human comparisons, RIA compared with TLC and GC □ Radioimmunoassay—triprolidine in animals and humans, comparisons with GC and TLC

Triprolidine hydrochloride {(*E*)-2-[3-(1-pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine monohydrochloride monohydrate} has been shown to have potent antihistaminic activity in animals (1–3) and to be clinically efficacious in humans (4–8). However, no detailed reports have appeared on the disposition of this drug, although [^{14}C]triprolidine was shown to be metabolized extensively *in vitro* by the guinea pig liver (9).

Circulating concentrations of triprolidine in human plasma following administration of oral therapeutic doses are very low and quite variable (10), necessitating the use of highly sensitive analytical techniques for their determination. Although TLC (11) and GC (12) procedures have been described for triprolidine, no data on the disposition of the drug were derived from these studies. A sensitive, quantitative TLC method for triprolidine was recently described by DeAngelis and coworkers (10), and the method was used to study some aspects of the disposition of this drug in rats and humans.

In this paper we present details of a specific radioimmunoassay (RIA) procedure for triprolidine which is capable of detecting 0.1 ng/mL of drug in plasma. This assay employs a gamma-emitting ^{125}I-radioligand, and thus is convenient for the analysis of large-scale triprolidine disposition studies. In the course of validating this method for human plasma analysis, the absolute oral bioavailability of triprolidine in the dog was determined by quantitative TLC, and some pharmacokinetic comparisons were made between rats, dogs, rabbits, and humans.

EXPERIMENTAL SECTION

The assay buffer used was 0.05 M Na_2HPO_4/NaH_2PO_4–0.15 M NaCl–0.01 M EDTA·Na_2–0.1% gelatin, pH 7.0. For separation of antibody-bound from free radiolabel, a dextran-coated charcoal suspension was prepared by dissolving 0.5 g of dextran[1] in 200 mL of ice-cold assay buffer, and adding 2 g of RIA-grade charcoal[2] to this solution. This suspension was stirred for at least 30 min at 0°C before use.

Hapten Synthesis—*2-Bromo-6-(4-toluoyl)pyridine* (*I*)—Butyllithium (50 mL, 1.65 M in hexane) was added under nitrogen to a stirred suspension of 2,6-dibromopyridine (19.5 g) in dry ether (200 mL) at −50°C. After 0.75 h a solution of 4-tolunitrile (10 g) in ether (50 mL) was added; stirring was continued at −50°C for 3 h. The mixture was allowed to warm to −30°C and

[1] T-70; Pharmacia Fine Chemicals, Piscataway, N.J.
[2] Schwarz-Mann, Orangeburg, N.Y.

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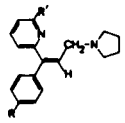
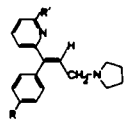
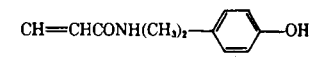
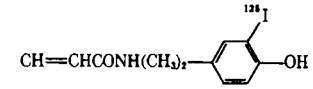
Table I—Structures and Cross-reactivities of Triprolidine and Related Compounds



IV and X



V-IX, XI and XII

Compound	R	R′	IC_{50}, ng/mL	Cross-reactivity, %[a]
IV	CH_3	$CH{=}CHCO_2H$	162.0	0.3
V	CH_3	$CH{=}CHCO_2H$	0.7	83.0
VI	CH_3	H	0.6	100.0
VII	CH_2OH	H	34.0	1.9
VIII	CO_2H	H	>1000.0	<0.05
IX	CF_3	H	1.12	49.1
X	CF_3	H	26.0	2.1
XI	CH_3		—	—
XII	CH_3		—	—

[a] Cross-reactivity of compound = (IC_{50} of triprolidine)/(IC_{50} of compound) × 100.

treated with HCl (200 mL, 2 M). The precipitated solid was collected, washed with water, and recrystallized from aqueous ethanol. The ketone formed colorless needles (12.2 g), mp 97–98°C. IR (KBr disk): 1660 cm^{-1} (aromatic ketone); ^{1}H-NMR ($CDCl_3$): δ 8.1–7.2 (m, 7, ArH) and 2.4 ppm (s, 3, Ar-CH_3).

Anal.—Calc. for $C_{23}H_{10}BrNO$: C, 56.52; H, 3.65; N, 5.07; Br, 29.0. Found: C, 56.48; H, 3.67; N, 4.93; Br, 28.53.

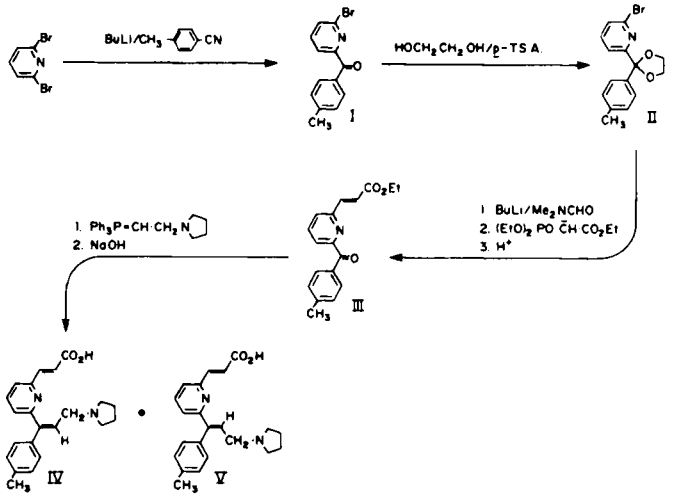
2-(6-Bromo-2-pyridyl)-2-(4-tolyl)-1,3-dioxolan (II)—A mixture of the above ketone (31 g), toluene (150 mL), ethylene glycol (10 mL), and *p*-toluenesulfonic acid (4 g) was heated at reflux for 6 h using a Dean–Stark trap. After cooling, the toluene solution was washed with excess ice-cold sodium bicarbonate solution, dried, and evaporated to 50 mL. Addition of petroleum ether to the warm solution induced crystallization of the dioxolan. From cyclohexane this formed colorless prisms (24.3 g), mp 110–112°C. ^{1}H-NMR ($CDCl_3$): δ 7.8–7.0 (m, 7, ArH), 4.1 (s, 4, $-CH_2-O-$), and 2.35 ppm (s, 3, Ar-CH_3).

Anal.—Calc. for $C_{15}H_{14}BrNO_2$: C, 56.25; H, 4.45; N, 4.4. Found: C, 56.48; H, 4.26; N, 4.29.

Ethyl (E)-3-[6-[(4-methylphenyl)carbonyl]-2-pyridinyl]propenoate (III)—A solution of II (3.5 g) in ether (100 mL) was cooled to −40°C and treated under nitrogen with butyllithium (10 mL, 1.64 M in hexane). After stirring at −50°C for 2 h, dimethylformamide (2 mL) was added. Two hours later the mixture was warmed to −30°C and treated with HCl (20 mL, 2 M). The organic phase was separated, washed with sodium bicarbonate solution, dried, and evaporated under reduced pressure. The residual aldehyde (2.5 g) was dissolved in 1,2-dimethoxyethane (10 mL) and added to a solution of the phosphonate carbanion produced from triethyl phosphonoacetate (2 g) and sodium hydride (0.22 g) in the same solvent. The mixture was stirred for 2 h, diluted with ether (25 mL), and treated with HCl (5 mL, 2 M). The organic phase was separated, washed with water, dried, and evaporated. The resulting oil was dissolved in ethanol (20 mL) containing concentrated HCl (3 mL) and water (3 mL). After heating on a steam bath for 10 min, the solution was diluted with ice water, rendered alkaline with sodium bicarbonate solution, and extracted with ether. After evaporation the residue was crystallized from cyclohexane as colorless platelets (1 g), mp 108–111°C. ^{1}H-NMR ($CDCl_3$): δ 7.65 (d, 1, J = 16 Hz, olefinic protons) and 6.85 ppm (d, 1, J = 16 Hz), implying *E* configuration.

Anal.—Calc. for $C_{18}H_{17}NO_3$: C, 73.2; H, 5.8; N, 4.75. Found: C, 72.7; H, 5.7; N, 4.59.

(E)-6-[(E)-3-(1-pyrrolinyl)-1-p-tolylpropenyl]-2-pyridineacrylic acid (V)—Butyllithium (10 mL, 1.64 M in hexane) was added under nitrogen to a stirred suspension of triphenyl-2-pyrrolidinoethylphosphonium bromide (13) (7.2 g) in dry toluene (75 mL). After 0.5 h, III (4.8 g) in toluene (50 mL) was added. The suspension, initially orange, became deep purple, then slowly faded to yellow during 2 h heating at 75°C. The cooled solution was diluted with ether (150 mL) and treated with HCl (50 mL, 2 M). The aqueous phase was separated, washed with ether, and basified with potassium carbonate (ice). Basic products were extracted into ether and immediately converted to oxalates by addition of oxalic acid (2.5 g) in ethanol (10 mL). The crude salts (5.1 g) melted at 148–155°C; it was shown by NMR that the new double bond had formed in the ratio 2:1, *Z*:*E*. The mixture of oxalates (3.7 g) was decomposed with sodium bicarbonate solution–ether. A solution of the bases in ethanol (100 mL) containing sodium hydroxide (20 mL, 1 M) was heated on the steam



Scheme I—Preparation of triprolidine hapten derivative.

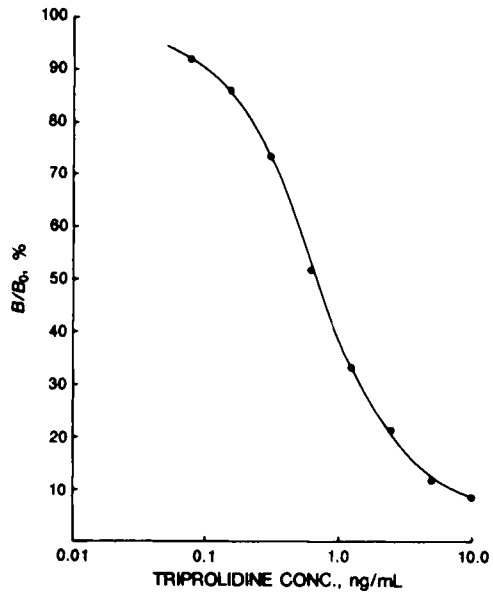


Figure 1—*Typical standard curve for triprolidine RIA in human plasma. Triprolidine was added to blank human plasma.*

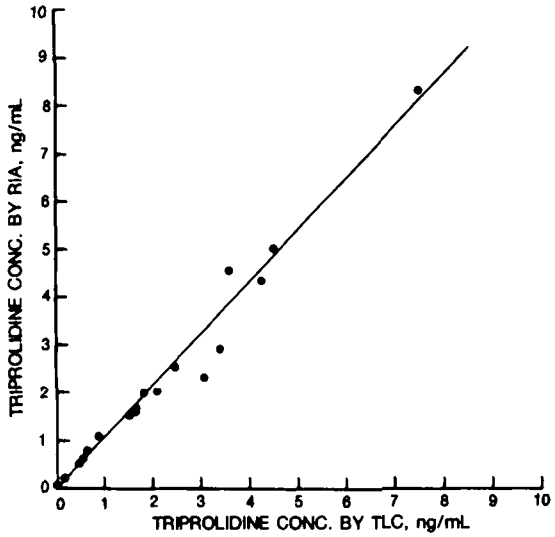


Figure 2—*Correlation of TLC and RIA analysis of triprolidine in human plasma samples.*

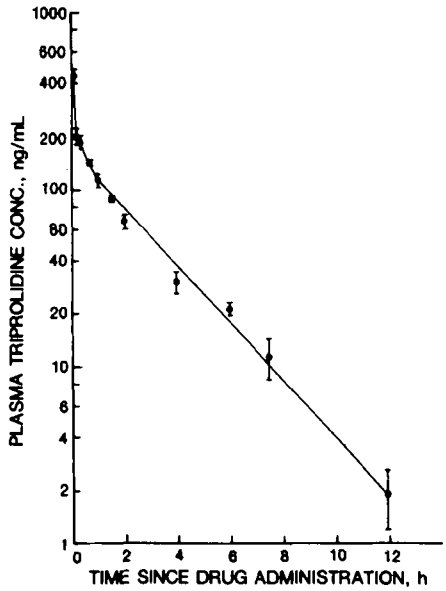


Figure 3—*Plasma disposition of triprolidine in male New Zealand White rabbits after an intravenous dose of 1 mg/kg of triprolidine hydrochloride. Triprolidine plasma concentrations are mean* ± SEM.

bath under reduced pressure for 5 min, neutralized with sulfuric acid (20 mL, 0.5 M), and finally evaporated to dryness. The solid residue was extracted with hot isopropyl alcohol (3 × 50 mL), and the combined filtrates were evaporated to dryness. The pinkish solid residue (2.5 g) was dissolved in hot ethyl acetate. Crystals (1.5 g), mp 160–162°C, were deposited on cooling; recrystallization from ethyl acetate gave the *E,Z*-isomer IV, mp 173–174°C (0.8 g). ^{1}H-NMR ($CDCl_3$) isolated olefinic proton at δ 6.35 ppm (t, 1). Concentration of the mother liquors afforded a second crop of crystals, mp 215–220°C (0.65 g), raised to 220–222°C (dec.) by recrystallization from isopropyl alcohol (0.55 g). ^{1}H-NMR ($CDCl_3$) isolated olefinic proton at δ 7.58 (t, 1), confirming that this was the *E,E*-isomer V.

Anal.—Calc. for $C_{22}H_{24}N_2O_2$: C, 75.86; H, 6.95; N, 8.05. Found: C, 75.57; H, 7.10; N, 8.03.

Conjugation of Acid V with Bovine Serum Albumin—Compound V (30 mg, 0.086 mM) was dissolved in ethanol (3 mL), and water (12 mL) was added. Bovine serum albumin (40 mg, 0.0057 mM) was added, the pH of the solution was adjusted to 5.5, and 1-ethyl-3-(3-dimethylaminoethyl)-carbodiimide hydrochloride[3] (25 mg, 0.161 mM) was added. This final solution was stirred overnight at room temperature, then was subjected to ultrafiltration[4] with water as eluant. After UV absorbance in the dialysate had returned to baseline levels, the solution of protein conjugate in the cell was lyophilized to give the drug–bovine serum albumin conjugate as a fluffy white solid (34 mg).

(E,E)-N-[2-(4-hydroxy-3-^{125}I-phenyl)ethyl]-3-[6-[1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl]-2-pyridinyl]propenamide (XII)—Compound V (30 mg, 0.086 mM) was dissolved in dimethylformamide (3 mL), and to this solution triethylamine (20 μL, 0.129 mM) was added. The solution was cooled in an ice bath, isobutyl chloroformate (15 μL, 0.108 mM) was added, and the reaction mixture was stirred at 0°C for 30 min. Tyramine (13 mg, 0.095 mM) in dimethylformamide (1 mL) was added, and the pH was adjusted to 8 with 1 M NaOH. The mixture was stirred overnight at 4°C; the addition of tyramine changed the initial pink/purple color to brown/orange. Solvents were removed under reduced pressure, and the residue was purified by preparative TLC on silica gel plates[5] developed in $CHCl_3$–CH_3OH–NH_4OH (70:30:1). The UV-absorbing band at R_f 0.73, which gave a slowly developing blue color when sprayed with ferric chloride–potassium ferricyanide, yielded the amide XI as an oil (13.6 mg) on elution with 10% CH_3OH–$CHCl_3$.

Amide XI was iodinated in the phenolic moiety by the method of Hunter and Greenwood (14). Compound XI (2 μg) in ethanol (10 μL) was mixed with 0.5 M Na_2HPO_4–NaH_2PO_4, pH 7.4 (25 μL). To this solution was added carrier-free [^{125}I]NaI[6] (1 mCi in 10 μL of NaOH solution) followed by chloramine-T hydrate (10 μg in 10 μL of 0.05 M phosphate buffer). The mixture was stirred for 30 s at room temperature, then sodium metabisulfite (25 μg in 25 μL of 0.05 M phosphate buffer) was added to stop the reaction. Preparative TLC of the reaction mixture on silica gel[5], as above, gave one major radioactive band at R_f 0.77 when radioscanned[7]. The iodinated compound was eluted from the silica with ethanol (13 mL), and the resulting solution was stored at −20°C until required. The approximate yield of iodinated amide XII was 420 μCi.

Immunization Procedure—Male New Zealand White rabbits received a primary immunization of 1 mg of bovine serum albumin-drug conjugate in 1 mL of 0.9% NaCl emulsified with 1 mL of Freund's complete adjuvant[8]. This dose was administered as two intramuscular (vastus lateralis) and eight subcutaneous (along the sides of the dorsal column) injections of 0.2 mL each. At intervals of 2, 4, and 6 weeks following the primary immunization, and at monthly intervals thereafter, booster immunizations of 0.5 mg of immunogen in 0.5 mL of saline, emulsified with 0.5 mL of Freund's complete adjuvant, were administered at multiple subcutaneous sites. Following the second and all subsequent booster immunizations, blood samples were collected from the central ear artery, and serum was collected and stored at −20°C until checked for the presence of antibodies. The antiserum used for the work described in this paper was obtained after 3 months of immunization.

Radioimmunoassay Procedures—All assay points were determined in duplicate; the results were then averaged. All assays were established in 12 × 75-mm polystyrene tubes[9].

Antiserum Titer Determinations—Various dilutions of rabbit antiserum in assay buffer (0.7 mL) were incubated with ^{125}I-triprolidine (50,000 cpm when freshly prepared, in 0.2 mL of assay buffer) and additional assay buffer (0.1 mL) in polystyrene tubes (12 × 75 mm) at 0°C for 2 h. Tubes containing only ^{125}I-triprolidine (0.2 mL) and buffer (0.8 mL) served as total count and nonspecific binding (background tubes). After 2 h, a dextran-coated charcoal suspension (0.5 mL) was added to all assay tubes, except total count tubes, and the contents were vortex-mixed and incubated at 0°C for 5 min. All charcoal-treated tubes were centrifuged at 4°C (≥1000×*g* for 10 min); the supernatants, containing the antibody-bound ^{125}I-triprolidine were decanted into clean polystyrene tubes, and the radioactivity was quantitated in a gamma[10] counter. Radioactivity bound by each antiserum dilution was expressed as percentage of total counts added to each assay tube. The antiserum titer was defined as that dilution which bound 40% of added radioligand.

Radioimmunoassay of Plasma Samples—Plasma samples from the various species were assayed directly, *i.e.*, without any prior extraction from the plasma. This procedure was similar to that given above, except that the assay incubation mixtures consisted of ^{125}I-triprolidine (0.2 mL) antiserum at the appropriate dilution (0.7 mL), and triprolidine standard solutions (0.1 mL) in blank plasma covering the range 0.078–10.0 ng/mL (7.8–1000 pg actual mass per tube). In addition to total count and nonspecific binding tubes, tubes containing antiserum (0.7 mL), ^{125}I-triprolidine (0.2 mL), and assay buffer (0.1 mL) served as maximum binding (B_0) tubes. Incubation and work-up procedures were as above. After quantitation of radioactivity, standard curves were expressed as percent B/B_0 against standard concentration on linear-log

[3] Sigma Chemical Co., St. Louis, Mo.
[4] Amicon cell with PM 10 membranes; Amicon Corp., Danvers, Mass.
[5] Merck silica gel GF_{254} 0.25 mm; E. Merck and Co., Elmsford, N.Y.
[6] Amersham Corp., Arlington Heights, Ill.
[7] Berthold, Model 2760; Beta Analytical Inc., Coraopolis, Pa.
[8] Difco Laboratories, Detroit, Mich.
[9] Walter Sarstedt Inc., Princeton, N.J.
[10] Model 5260 Autogamma Counter; Packard Instrument Co. Inc., Downers Grove, Ill.

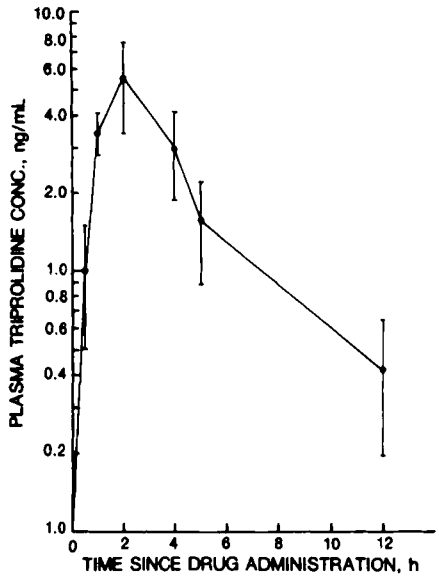


Figure 4—*Plasma triprolidine concentration–time curves in three normal human subjects after a single oral dose of 2.5 mg of triprolidine hydrochloride. Triprolidine concentrations are mean ± SEM.*

scales, where B is the antibody-bound radioactivity in the presence of a given standard concentration.

Cross-reactivity Studies—These studies were carried out as for the RIA except that in addition to the triprolidine standard curve, curves were established with the triprolidine metabolites and related compounds (IV–XI) shown in Fig. 2, and with some structurally unrelated compounds which might be administered concomitantly as medications (pseudoephedrine, guaifenesin, codeine, dextrorphan, aspirin, and acetaminophen). Percentage cross-reactivities were expressed as:

$$(IC_{50} - \text{Compound})/(IC_{50} - \text{triprolidine}) \times 100$$

where IC_{50} is the concentration required to inhibit binding of radioligand to antiserum by 50% (15).

Quantitative Thin-Layer Chromatographic Analysis of Triprolidine—This analytical method was used as published (10) with some modifications. Briefly, the drug was extracted into benzene, the organic layer was separated by centrifugation, and a portion was concentrated by evaporation. The residue was dissolved in chloroform–methanol (85:15, 80 μL) and the entire volume was spotted on a silica gel plate. The plate was developed in chloroform–methanol–ammonium hydroxide (91:8:1) to a height of 11 cm. After spraying with 2 M ammonium bisulfate, the induced fluorescence of the drug was quantitated at an excitation wavelength of 300 nm (cut-off filter 400 nm). The recovery of the drug from plasma across a 1–8-ng/mL concentration range was 95%.

Human Disposition Studies—Three healthy volunteers (2 male, 1 female; mean weight, 68.0 kg) who were not receiving any drug therapy, fasted overnight (12 h) and, at 8 a.m., the next morning, ingested a tablet containing 2.5 mg of triprolidine hydrochloride[11]. No food was consumed until 2 h after drug administration. Plasma was harvested and stored as above. After 1 week, each dog received the same dose of drug by the alternate route of administration, and blood samples were collected in the same manner.
stored at −20°C until analyzed for triprolidine by RIA or TLC.

Animal Disposition Studies—*Dogs*—Two male beagles were fasted overnight. The next morning, both dogs received 1-mg/kg doses of triprolidine hydrochloride, one as an oral dose (gelatin capsule) and the other as intravenous injection (saphenous vein) in isotonic saline (2 mL). Blood samples were collected into EDTA-containing evacuated tubes[12] prior to drug administration and at 0.08, 0.17, 0.33, 0.5, 0.66, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after drug administration. Plasma was harvested and stored as above. After 1 week, each dog received the same dose of drug by the alternate route of administration, and blood samples were collected in the same manner.

Rabbits—Three male New Zealand White rabbits were fasted overnight before intravenous administration of triprolidine hydrochloride (1 mg/kg) in isotonic saline (2 mL) into the marginal ear vein. Blood samples were collected from the contralateral marginal ear veins prior to and at 0.03, 0.17, 0.33, 0.67, 1, 1.5, 2, 4, 6, 7.5, and 12 h after drug administration. Blood samples were processed as described above for dogs.

[11] Actidil; Burroughs Wellcome Co., Greenville, N.C.
[12] Vacutainer; Becton, Dickinson & Co., Rutherford, N.J.

Table II—Intravenous and Oral Administration of Triprolidine (1 mg/kg) to Beagle Dogs

Time, h	Plasma Concentration, ng/mL			
	Dog 46579		Dog 46965	
	Intravenous	Oral	Intravenous	Oral
0.08	184.8	0	290.5	0
0.16	146.1	1.7	225.9	0
0.33	117.6	2.5	211.5	2.0
0.50	94.3	3.5	173.4	6.6
0.67	86.0	4.3	118.2	5.7
1	55.8	4.8	100.1	4.3
1.5	32.6	3.2	50.2	2.8
2	28.4	2.4	35.7	2.2
4	10.4	1.6	10.4	0.9
6	3.8	0.6	3.6	0.3
8	2.7	0	1.9	0
12	1.0	0	0.7	0
AUC_0^{12}, ng·h/mL	211.7	8.7	305.1	11.2
Bioavailability, %		4.1		3.7

Pharmacokinetic Analysis—The mean plasma concentration–time data from the present human and rabbit experiments and a previous rat study (10), and the individual data sets from the dog experiments were analyzed pharmacokinetically by the computer program NONLIN (16), which iteratively minimizes the sum of the squared deviations between observed and computed plasma concentrations at the time points following drug administration. The volume of distribution, Vd_β, was calculated from the intravenous data using the equation $Vd_\beta = \text{Dose}/\text{AUC} \times \beta$, where AUC is the area under the plasma concentration–time curve from time zero to the last plasma sampling time, and β is the rate constant describing the terminal exponential phase of drug elimination from the plasma. Absolute bioavailability was determined from the ratio of the AUC values following oral and intravenous drug administrations.

RESULTS AND DISCUSSION

Synthesis of Hapten and Radioligand—Drug molecules are normally not immunogenic *per se*, and require prior coupling to a macromolecular carrier in order to elicit antibody production in animals (17, 18). A hapten derivative of triprolidine, suitable for coupling to protein, was prepared as shown in Scheme I. Starting from 2,6-dibromopyridine (I), the toluoyl derivative (II) was readily prepared and protected as the dioxolan (III), while the remaining bromine atom was replaced in a three-step procedure leading to the ethyl acrylate derivative (III). A Wittig reaction on the ketone moiety, followed by hydrolysis gave the acryloyl analogue of triprolidine (V, *E,E*-isomer) along with a larger amount of the *E,Z*-isomer (IV). These isomers were readily separated and purified by fractional crystallization. The triprolidine analogue (V) was successfully conjugated to bovine serum albumin by carbodiimide-mediated peptide bond formation, and the resulting drug–protein conjugate, after purification by ultrafiltration, was used for rabbit immunization experiments.

A convenient, gamma-labeled radioligand was prepared by coupling of tyramine to the acryloyl-triprolidine derivative (V) by the mixed anhydride method, followed by iodination of the phenolic moiety of the resulting amide XI with [^{125}I]NaI by the chloramine-T method of Hunter and Greenwood (14). This compound (XII, ^{125}I-triprolidine) was well-recognized by the antiserum, with >80% of 50,000 cpm of freshly prepared radioligand being bound by excess antiserum.

Antiserum Production—All three rabbits immunized with the bovine serum albumin–triprolidine conjugate produced antisera capable of binding ^{125}I-triprolidine after 2–4 months of the immunization program. The titer of these sera was sensitive to the presence of heterologous animal serum added to the incubation mixture. Thus, serum 830/2, which was employed for all of the work reported in this paper, exhibited apparent titers (40% total ^{125}I-triprolidine bound) of 1:140,000, 1:70,000, and 1:50,000 in the presence of human, dog, and rat (Long-Evans and Sprague-Dawley) plasma, respectively. The possibilities that these differences may be due to species variations in plasma proteins or to the presence of endogenous substances which are recognized by the triprolidine antiserum, have not been explored.

Assay Sensitivity, Accuracy, and Precision—Figure 1 is a typical triprolidine RIA standard curve for the assay in human plasma. The sensitivity of this system, defined as the concentration of triprolidine required to inhibit binding of radiotracer to antiserum by 10%, is ~0.1 ng/mL (0.01 ng actual weight). Clearly, this sensitivity could be increased by manipulation of the weight of radioligand and titer of antiserum used in the assay. Multiple analyses (on

different days) were conducted using a series of control triprolidine samples, prepared by addition of the hydrochloride salt to pooled blank human plasma to give various free base concentrations. The concentrations added were 0.2 (n = 25), 2.0 (n = 26), and 20.0 (n = 24) ng/mL; mean concentrations ($\pm SD$) measured were 0.21 (±0.05), 2.15 (±0.23), and 20.35 (±1.81), respectively. The highest control (20 ng/mL) was diluted 1:10 with blank plasma before analysis. These values therefore reflect interassay accuracy and precision data from averaged pairs of data points. The close agreement of the mean measured triprolidine concentrations with those actually added indicates the accuracy of the method. Standard deviations on the 2- and 20-ng/mL control samples indicate roughly 10% CV, while this value rises above 20% for the 0.2-ng/mL sample. Thus, at concentrations clearly above the sensitivity limit of the procedure, the assay method has good precision.

Assay Specificity—The specificity of the antiserum was examined by studying the relative abilities of triprolidine and a series of its metabolites and some related compounds to displace ^{125}I-triprolidine from the antiserum (Table I). The hapten derivative (V), which was coupled to bovine serum albumin to provide the immunogen, is almost as effective as triprolidine in displacing the radiolabel, but the *cis* isomer (IV) is almost 28-fold less effective. Although the *cis* isomer of triprolidine itself was not available for study, the ability of the antiserum to distinguish stereoisomers about the central double bond of the triprolidine parent structure was confirmed by examination of the cross-reactivities of the trifluoromethyl analogues (IX and X). The direct trifluoromethyl analogue of triprolidine (IX) cross-reacted extensively with the antiserum (49%), which indicates that the trifluoromethyl substituent is quite similar, antigenically, to the methyl substituent in triprolidine. However, the *cis*-trifluoromethyl isomer (X) cross-reacted ~25-fold less (2%), again confirming the recognition of stereochemistry around the central double bond. Several other stereospecific drug antisera have been developed recently (19-21), emphasizing that the specific properties of antisera extend beyond recognition of gross stereoelectronic changes to that of relatively fine differences in stereochemistry.

The aromatic acid (VIII), which has been identified as a major metabolite of triprolidine in animals and humans (9), exhibited only 15% inhibition of binding at 1000 ng/mL, indicating very low cross-reactivity (≪0.5%). The structural change in the benzylic alcohol (VII), also a metabolite of triprolidine, was also well-recognized, leading to a relatively low cross-reactivity value of 1.9% relative to triprolidine. A number of compounds which were not structurally related to triprolidine, but which may be coadministered as medications with the drug, were also examined. Codeine, pseudoephedrine, aspirin, acetaminophen chlorpheniramine, guaifenesin, and dextrorphan all showed little inhibition of binding (2–8%) at a concentration of 10 μg/mL. Surprisingly, chlorpheniramine did have weak (2.5%), but measurable, cross-reactivity in the triprolidine radioimmunoassay.

Assay Validation—The RIA procedure for triprolidine in human plasma was validated by comparative analysis of a group of human plasma samples by RIA and by a previously published quantitative TLC method (10). The samples were collected from volunteers at various times after the ingestion of a single dose (2.5 mg) of triprolidine hydrochloride (Fig. 2). The regression equation obtained was, RIA = 1.076 × TLC − 0.071. An excellent correlation (r = 0.985) and good agreement (slope = 1.076) was found for these two analytical methods, confirming the validity of the RIA procedure for triprolidine analysis in human plasma.

To illustrate the utility of the assay procedure, several experiments involving the oral administration of triprolidine hydrochloride to dogs, rats, guinea pigs, rabbits, and humans were established. In the case of dogs and rats, direct analysis of triprolidine by RIA without extraction was unsuccessful due to the failure of plasma samples to dilute in a manner parallel to the triprolidine assay standard curve. Markedly increased apparent triprolidine concentrations were obtained on each dilution, perhaps as a result of cross-reaction from an unknown metabolite that may have a displacement curve nonparallel to that of triprolidine. These samples could be analyzed readily by RIA after a preliminary benzene extraction of triprolidine from the plasma (10). Plasma samples from guinea pigs, rabbits, and humans could be analyzed without prior extraction at dilutions which placed them clearly in the linear portion of the RIA standard curve.

The disposition of triprolidine in three male New Zealand White rabbits, following intravenous injection of 1 mg/kg of triprolidine hydrochloride, was determined by the present RIA procedure (Fig. 3). The plasma concentration–time data, as analyzed by the NONLIN computer program (16), fitted well to a two-compartment open model of drug disposition. The rapid disposition phase was characterized by a rate constant, α = 12.3 h^{-1} ($t_{1/2,\alpha}$ = 0.06 h), while the terminal elimination phase was described by the rate constant, β = 0.41 h^{-1} ($t_{1/2,\beta}$ = 1.68 h). Tissue distribution in the rabbit appeared to be extensive, as indicated by the relatively large volume of distribution (Vd_β = 4.66 L/kg). The $t_{1/2,\beta}$ value for triprolidine in the rabbit (1.68 h) is comparable to the $t_{1/2}$ in humans (10).

Table III—Species Comparison of Kinetic Parameters

Parameter	Rabbit[a]	Human[b]	Dog[a]	Rat[a,c]
α, h^{-1}	12.3	—	1.3	6.0
β, h^{-1}	0.41	0.31	0.45	0.79
$t_{1/2,\alpha}$, h	0.06	—	0.40	0.12
$t_{1/2,\beta}$, h	1.68	2.27	1.5	0.88
Vd_β, L/kg[c]	4.7	2.6	9.3	9.6
AUC, ng·h/mL	462.0	39	259	134

[a] Intravenous 1-mg/kg injections of triprolidine hydrochloride. [b] Total oral dose of 2.5 mg of triprolidine hydrochloride; Vd_β term is actually Vd_β/F. [c] Parameters derived from data in Ref. 10.

The disposition of triprolidine in the plasma of three healthy subjects (two males and one female) following ingestion of 2.5 mg of triprolidine hydrochloride in a proprietary tablet preparation was examined, using RIA for analysis of plasma drug concentrations. The mean plasma concentration–time data are plotted in Fig. 4. In these three subjects, peak triprolidine concentrations ranged from 3.1 to 9.4 ng/mL at 1–2 h after drug administration. Triprolidine could still be detected in the plasma by RIA 24 h after drug ingestion, but levels at that time were close to the sensitivity limit of the method. The concentration–time data gave a good fit to a one-compartment open model of drug disposition, with first-order absorption. The apparent elimination half-life of 2.3 h in these subjects was similar to previously reported values for triprolidine in humans (10). The value for Vd_β/F is large (2.6 L/kg), also implying extensive tissue distribution or drug metabolism, although knowledge of the value of F (fraction of dose bioavailable) is necessary before the true volume of distribution can be calculated.

Although triprolidine could not be determined in dog plasma by the direct RIA procedure, TLC analysis provided data on the disposition of triprolidine in the dog for comparison with other species. Plasma concentrations of triprolidine in two beagles following separate oral and intravenous administrations of 1-mg/kg doses are presented in Table II. Observed peak plasma levels following oral administration (4.8 and 6.6 ng/mL) were 12–26-fold lower than concentrations at the same time after intravenous drug administration. The trapezoidal rule (22) was used to determine the area under the plasma concentration–time curves (AUC), and the absolute bioavailability of the oral dose was expressed as the percentage ratio of AUC values following oral and intravenous drug administrations (23). The bioavailability of triprolidine in these dogs was very low (3.7 and 4.1%), suggesting extensive first-pass metabolism of the drug in the liver and/or gut after oral administration. However, the data available on triprolidine, both from the present study and in general, do not exclude the possibility that this drug is poorly absorbed by the dog after oral administration, which could also result in low oral bioavailability. The intravenous data fitted well to a two-compartment open model of drug disposition and the kinetic parameters emanating from this analysis are presented in Table III. The mean $t_{1/2,\beta}$ and Vd_β values of 1.5 h and 9.2 L/kg, respectively, are comparable with similarly derived parameters in the rabbit in this study and the rat (10) in a previous study (Table III).

In this paper we have described a new RIA procedure for triprolidine, which is sufficiently sensitive and specific for use in the analysis of the drug in human plasma samples up to 24 h after ingestion of therapeutic oral doses. The method has also been used to provide pharmacokinetic data for triprolidine in humans and rabbits for comparison with similar data in dogs and rats. This procedure is currently being used in studies of the pharmacokinetics of triprolidine in humans after oral administration of immediate- and sustained-release drug formulations.

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Novel Approach to Zero-order Drug Delivery *Via* Immobilized Nonuniform Drug Distribution in Glassy Hydrogels

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Abstract □ A novel approach to zero-order drug delivery from glassy hydrogel matrices *via* an immobilized, sigmoidal, initial drug distribution has been developed. The method utilizes a controlled-extraction process on initially dry, drug-loaded hydrogels to generate an inflection-point-containing drug concentration profile followed by a vacuum freeze-drying step to rapidly remove the swelling solvent and immobilize *in situ* a nonuniform drug distribution. The drug release from such a system generally exhibits typical zero-order characteristics similar to that of a membrane-reservoir device. However, a saturated reservoir of active ingredient as in the membrane-reservoir device is not required because the constant release is achieved *via* an initially nonuniform concentration distribution instead of the constant activity in a reservoir. The applicability of the present concept and process has been demonstrated experimentally with the release of oxprenolol hydrochloride from hydrogel beads based on 2-hydroxyethyl methacrylate polymerized with a polymeric cross-linking agent.

Keyphrases □ Glassy hydrogels, oxprenolol hydrochloride—nonuniform drug distribution □ Oxprenolol hydrochloride—glassy hydrogels, nonuniform drug distribution □ Drug delivery systems—oxprenolol hydrochloride, glassy hydrogels

Diffusion-controlled polymeric delivery systems are finding increasing applications in the area of controlled release of pharmaceuticals (1–3). To achieve optimal therapeutic effects, especially for drugs with short physiological half-lives, it is often desirable to have a zero-order (or constant-rate) drug delivery (4). Unlike membrane-reservoir devices, monolithic systems where the drug is uniformly dissolved or dispersed in a polymer matrix generally do not exhibit zero-order release behavior. Instead, a release rate that continuously diminishes with time is observed (5, 6). This is a consequence of the increased diffusional distance and decreased area at the penetrating diffusion front. In addition to geometry factors (7), methods that approach zero-order release from monolithic matrices generally involve the introduction of either a constant rate of surface erosion much larger than the drug diffusion rate in the polymer matrix (8–10) or a constant rate of solvent front penetration (the so-called case II swelling) much smaller than the drug diffusion rate in the swollen region (11, 12). The applicability of these systems may be further limited by the need to maintain a constant surface area at the erosion or penetrating solvent front.

An important concept which has not been explored is the approach to zero-order drug release from a glassy polymer matrix having a specific nonuniform initial drug concentration distribution. Hydrogel polymers are unique for this application in that they are glassy in the dry state and capable of immobilizing any nonuniform drug distribution introduced prior to the dehydration step. In the presence of water, hydrogels can absorb a significant amount of water to form an elastic gel and, at the same time, release the dissolved drug by diffusion through the swollen region (13, 14).

This paper reports a method for immobilizing such a nonuniform drug concentration distribution in glassy hydrogel beads and the resulting zero-order drug release behavior. A

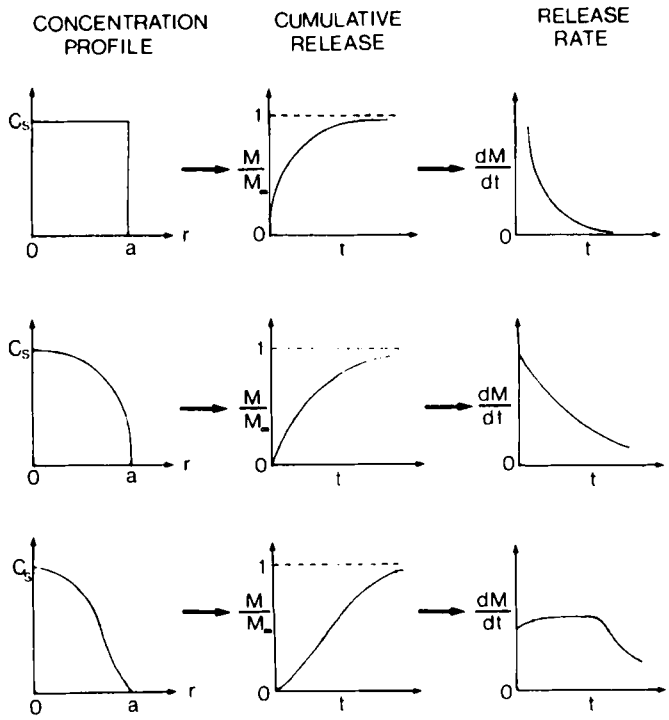

Figure 1—*Theoretical profiles illustrating the characteristics of drug release from spherical matrices as a function of the initial drug concentration distribution.*

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