appeared to be mutagenic (60 revertants/15 μg). It should be stressed, however, that these are preliminary studies and would have to be confirmed using other tester strains of *Salmonella typhimurium*.

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Plasma Levels of a Novel Antidysrhythmic Agent, Meobentine Sulfate, in Humans as Determined by Radioimmunoassay

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Received July 27, 1981, from the * *Wellcome Research Laboratories, Research Triangle Park, NC 27709;* and the ‡ *Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.* Accepted for publication September 21,1981.

Abstract □ A radioimmunoassay for the quantitation of meobentine sulfate, a novel antidysrhythmic and antifibrillatory agent in biological fluids, is described. Antisera were raised in rabbits in response to immunization with a conjugate of bovine serum albumin and a meobentine analog with a propionic acid sidechain *ortho* to the methoxyl group. These antisera have low affinities for *N*- and *O*-desmethylmeobentine metabolites, which show less than 5% cross-reaction in radioimmunoassay procedures employing either tritiated or radioiodinated radioligands. The radioimmunoassay using [^{125}I]meobentine was capable of detecting <0.4 ng/ml (40-pg mass) of meobentine. This assay was used to demonstrate the absorption of meobentine in humans after oral administration and also permitted studies of meobentine sulfate disposition in human plasma following two (2.5 and 5 mg/kg) oral doses. Mean peak meobentine concentrations in plasma occurred 3 hr postdose in both cases and were 230 and 451 ng/ml following the 2.5- and 5-mg/kg doses, respectively. The approximate mean terminal half-life after all treatments was 12 hr.

Keyphrases □ Meobentine sulfate—plasma levels in humans, radioimmunoassay □ Bioavailability—plasma levels of meobentine sulfate in humans determined by radioimmunoassay □ Radioimmunoassay—plasma levels of meobentine sulfate in humans

Meobentine sulfate [bis-(*N*-4-methoxybenzyl-*N'*-*N''*,dimethylguanidinium)sulfate] possesses marked antidysrhythmic properties against arrhythmias induced by ouabain and those induced by coronary artery ligation in dogs (1). It has been demonstrated that meobentine causes a significant incidence of spontaneous recovery from electrically induced fibrillation in the dog[1]. The electrophysiological properties of meobentine have been studied (2). Although very close in structure to the hypotensive agent, bethanidine (3), meobentine is not a neuronal blocking agent and thus, does not decrease blood pressure in animals when administered intravenously at effective antidysrhythmic doses (1). In this respect, meobentine also appears to be superior to the quaternary ammonium compound bretylium tosylate, which is indicated only for treatment of life-threatening ventricular arrhythmias (4) due to its severe hypotensive side effects.

The clinical safety and efficacy of meobentine sulfate when administered to humans by oral and parenteral routes are currently under study. Pharmacokinetic studies of meobentine in animals and humans, which are needed to facilitate the evaluation of meobentine in current clinical trials, require adequately sensitive and specific procedures for the determination of the drug in body fluids. Earlier gas and thin-layer chromatographic techniques lacked sensitivity, while administration and quantitation of radiolabeled meobentine is impractical for extensive pharmacokinetic studies in humans.

[1] W. Wastila *et al.*, submitted to *J. Pharm. Pharmacol.*

0022-3549/82/0600-0665$01.00/0

Radioimmunoassay methods have been applied increasingly to the quantitation of drugs (5, 6). This report describes the development of sensitive and specific radioimmunoassay procedures for meobentine, employing tritium or radioiodine ligands. The sensitivity limit for the procedure using the ^{125}I-labeled meobentine was <0.4 ng/ml (40-pg mass), while interference with desmethyl metabolites of the drug was minor. These analytical procedures were used to demonstrate the absorption of meobentine after oral administration to healthy subjects and to explore the relationship of plasma concentrations and relative oral bioavailability to the oral dose administered.

EXPERIMENTAL

Chemicals—Thin-layer chromatography (TLC) employed micropolyamide on a nylon support[2] or silica gel[3] on a glass support[4]. Radiochemical purities were determined by scanning thin-layer plates on a radiochromatogram scanner[5]. Bovine serum albumin[6] isobutyl chloroformate[6], Freund's complete adjuvant[7], [^{125}I]sodium iodide[8], and polyethylene glycol 6000[9] were obtained commercially. Methyl isothiocyanate, 4-hydroxybenzaldehyde, and 5% palladium on charcoal were obtained from a single supplier[10], as were chloramine-T and *N*-nitrosomethyl urea[11]. Beta-emitting radionuclides (^{3}H and ^{14}C) were quantitated in scintillation fluid[12] in a scintillation counter[13]. Gamma radiation was quantitated in a spectrometer[14]. *N*-Desmethylmeobentine was a gift[15], as also were guanethidine[16], clonidine[17], guanabenz[18], propranolol[19], isoproterenol[20], and procainamide[21].

Immunogen Preparation—*3-(5-Cyano-2-methoxyphenyl)-propionic Acid (II)*—A mixture of 10 g of 3-(5-bromo-2-methoxyphenyl)propionic acid (I) (7), cuprous cyanide (15 g), and pyridine (20 ml) was heated with stirring in a bath at 200° for 4 hr. The cooled material was triturated with water (150 ml) and filtered, and the solid mass of copper salts was extracted with warm dilute sodium hydroxide solution (2 × 100 ml). Acidification of the combined aqueous filtrates gave 7 g of the crude acid (II) mp 142–146°. Recrystallization from aqueous ethanol and then from benzene raised the mp to 147–148° (4.7 g).

Anal.—Calc. for $C_{11}H_{11}NO_3$: C, 64.39; H, 5.37; N, 6.83. Found: C, 63.53; H, 5.23; N, 6.32.

3-(5-Aminomethyl-2-methoxyphenyl)propionic Acid (III)—A mixture of 4.5 g of the cyano-acid (II) in ammonium hydroxide solution (35%; 30 ml) containing a nickel catalyst[22] (~2 g) was stirred under hydrogen at 10^7 N/m^2 and 100° for 4 hr. The mixture was filtered and the filtrate was evaporated to dryness. The resulting greenish solid was extracted with warm water (3 × 10 ml) and the extracts were freed from nickel salts by passage of H_2S followed by filtration. The amino acid (III), isolated by evaporation *in vacuo*, crystallized from aqueous ethanol in colorless platelets (2.5 g) mp 218–219° (dec).

Anal.—Calc. for $C_{11}H_{15}NO_3$: C, 63.2; H, 7.2; N, 6.7. Found: C, 63.09; H, 7.32; N, 6.78.

3-[2-Methoxy-5-(N′,N″-dimethylguanidinomethyl)phenyl]-propionic Acid (VI)—Methyl isothiocyanate (0.92 g) in ethanol (40 ml) was added to a solution of 2.4 g of the amino acid (III) in 1 *M* NaOH solution (12 ml). Sulfuric acid (0.5 *M*, 12 ml) was added 24 hr later, followed by acetone (100 ml). The solution was filtered from inorganic material and treated with methyl iodide (10 ml). After 1 hr the mixture was evaporated *in vacuo* and the residue was treated with 30% aqueous methylamine solution (40 ml). Gentle warming on a steam bath for 1 hr followed by evaporation to dryness yielded the product as a waxy solid. Recrystallization from isopropyl alcohol and then from water afforded colorless needles, mp 201–204° (0.9 g).

Anal.—Calc. for $C_{14}H_{21}N_3O_3$: C, 56.56; H, 7.80; N, 14.14; $1H_2O$, 6.06. Found: C, 56.23; H, 8.13; N, 13.87; loss at 100°/VAC 6.06.

3-[2-Methoxy-5-(N′,N″-dimethylguanidinomethyl)phenyl]propionic Acid Bovine Serum Albumin-Conjugate—A solution of the guanidino–propionic acid (VI) (0.05 g, 0.168 m*M*) in a warm, 20-ml mixture of dimethyl sulfoxide–dimethylformamide (2:1) was cooled to 0°. Triethylamine (0.035 liter, 0.252 m*M*) and isobutyl chloroformate (0.025 liter, 0.190 m*M*) were added and the solution was stirred for 1 hr at 0°. This cold (0°) solution of the mixed anhydride was added dropwise to a solution of bovine serum albumin (0.080 g, 0.065 m*M* ϵ-amino lysine residues) in 160 ml of a 0.1 *M* sodium bicarbonate solution at 0° and the mixture was stirred overnight at 4°. After filtering insoluble material, the solution was pressure dialysed through a membrane-containing filter unit[23] (50 psi, 2 liter H_2O), and finally lyophilized, yielding an amorphous powder (0.075 g).

Synthesis of Radiolabeled Compounds—*[^{3}H]bis(N-4-methoxybenzyl-N′,N″-dimethylguanidinium)sulfate*—This compound was prepared by a supplier under contract, using general tritium exchange procedures. The specific activity of this compound was estimated to be 0.8 Ci/m*M*.

Bis[N-(3-iodo-125-4-methoxybenzyl)-N′,N″-dimethylguanidinium]-sulfate (VIII).

N-4-Hydroxybenzyl-N′,N″-dimethylguanidinium iodide (VII)—Prepared as described previously (8).

Bis[N-(3-iodo-125,4-hydroxybenzyl)-N′,N″-dimethylguanidinium]sulfate (VIII)—To VII sulfate (2.7 μg, 11.2 n*M*) in phosphate buffer (70 μl, 0.5 *M*, pH 7.7) contained in a small test tube was added carrier-free [^{125}I]sodium iodide (1.12 n*M*, 2 mCi) in 20 μl of dilute sodium hydroxide followed by a freshly prepared solution of chloramine-T (47.25 μg, 0.168 μ*M*) in phosphate buffer (25 μl, 0.05 *M*, pH 7.7). After 1 min of agitation, a freshly prepared solution of sodium metabisulfite (0.64 mg, 3.36 μ*M*) in phosphate buffer (100 μl, 0.05 *M*, pH 7.7) was added to stop the reaction. The entire reaction mixture was then applied to the origin of a micropolyamide TLC plate (0.1 mm, 20 × 20 cm) and allowed to dry.

Bis[N-(3-iodo-125,4-methoxybenzyl)-N′,N″-dimethylguanidinium]-sulfate (IX)—To the origin of the TLC plate containing the iodinated phenolic guanidine VIII was repeatedly applied an ether solution of diazomethane prepared from *N*-nitrosomethyl urea (5 g) (9). The plate was then developed in the system H_2O–triethylamine (100:1) and then allowed to expose an X-ray film. Radioiodine-containing material remaining at the origin was eluted with methanol (3 × 5 ml) and the solution was stored at −80°. The specific activity of the material obtained (1 mCi) was estimated to be 179 Ci/m*M*. Radiochemical purity was established by TLC on silica gel in the system *n*-butanol–methanol–water–acetic acid (4:20:20:10) in which the R_f (0.6) of the iodinated product was identical to that of unlabeled meobentine sulfate.

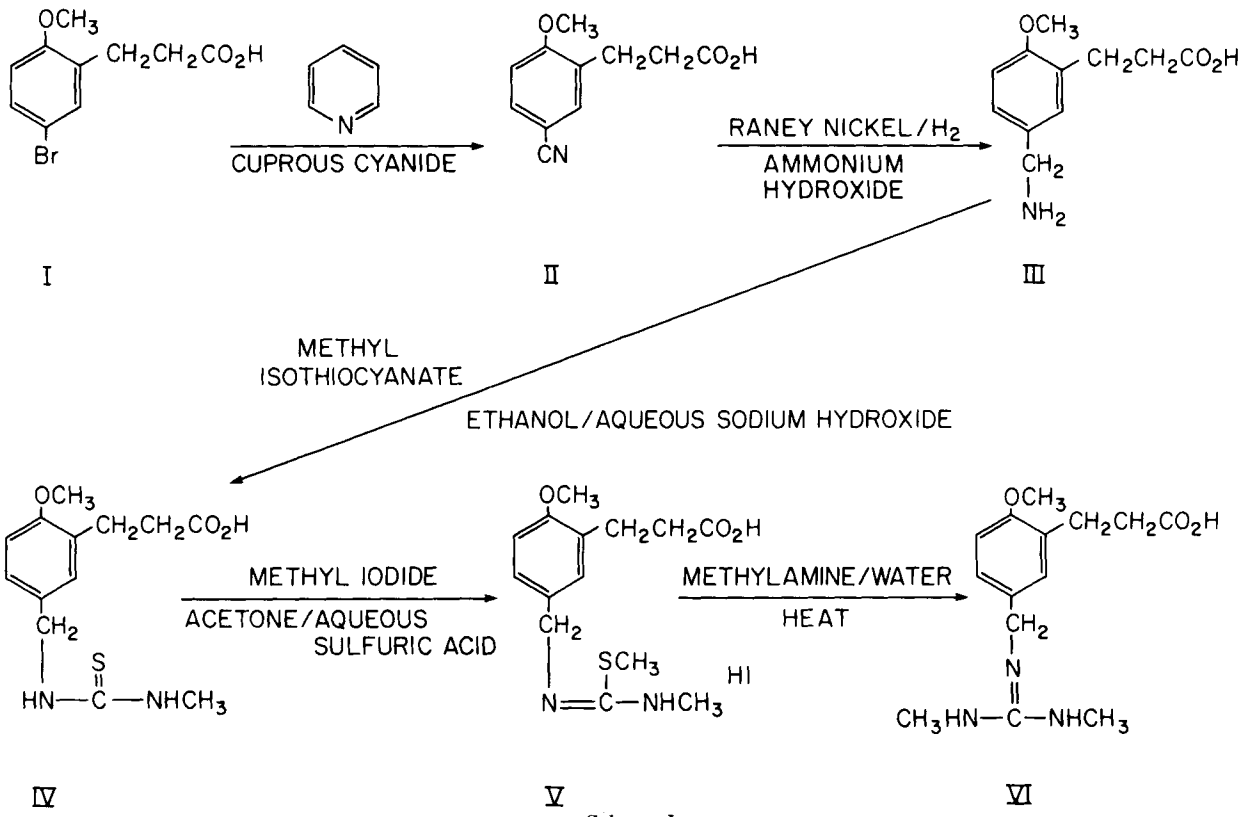
Immunization Procedures—Male New Zealand white rabbits received a primary immunization of bovine serum albumin-drug conjugate (1 mg) in 0.9% saline (1 ml) emulsified with Freund's complete adjuvant (1 ml) as two intramuscular (vastus lateralis) and eight subcutaneous injections (along each side of the dorsal column) 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 immunogen (0.5 mg) in saline (0.5 ml), emulsified with Freund's complete adjuvant (0.5 ml), were administered at multiple subcutaneous sites. Following the second and all subsequent booster immunizations, blood samples were collected from the central ear artery and the serum was separated and stored at −80°.

Clinical Studies—Six healthy subjects (three male and three female) each took 2.5 mg/kg of meobentine sulfate and then 5.0 mg/kg 1 week later. (One male subject did not complete the study at the higher dose.) The drug was administered in 100 ml of water. Plasma was sampled beforehand and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 24, 32, 48, and 56 hr afterwards. Samples were taken by collecting 5-ml venous blood into a lithium heparin tube which was immediately centrifuged. The separated plasma was then immediately removed and stored at −20° until assayed.

Radioimmunoassay Procedures—The assay buffer used throughout these studies was 0.05 *M* Na_2HPO_4, 0.15 *M* NaCl, and 0.01 *M* edetic acid,

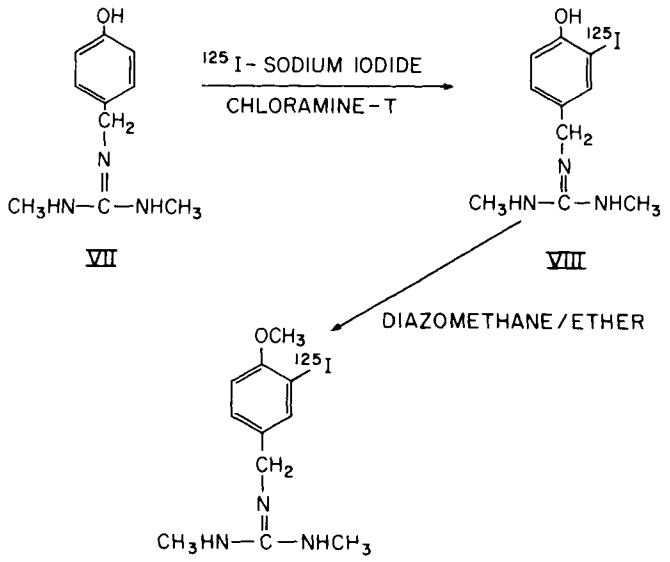
[2] Schleicher and Schull, Inc., Keene, N.H.
[3] GF_{254}, E. Merck and Co., Elmsford, N.Y.
[4] E. Merck and Co., Elmsford, N.Y.
[5] Berthold LB2760, Beta Analytical Inc., Coraopolis, Pa.
[6] BSA, Cohn Fraction V, Sigma Chemical Co., St. Louis, Mo.
[7] Difco Labs, Detroit, Mich.
[8] Amersham Corp., Arlington Heights, Ill.
[9] Fisher Scientific Co., Raleigh, N.C.
[10] Aldrich Chemical Co., Milwaukee, Wis.
[11] Eastman Organic Chemicals, Rochester, N.Y.
[12] Aquasol-2, New England Nuclear Corp., Boston, Mass.
[13] Model 2650, Packard Instrument Co., Downer's Grove, Ill.
[14] Model 5260 Autogamma, Packard Instrument Co., Downer's Grove, Ill.
[15] Dr. F. Copp, Wellcome Research Labs, Beckenham, U.K.
[16] Ciba-Geigy, Inc., Summit, N.J.
[17] Boehringer-Ingelheim, Ingelheim, West Germany.
[18] Wyeth Labs, Inc., Radnor, Pa.
[19] Ayerst Labs, New York, N.Y.
[20] Winthrop Labs., New York, N.Y.
[21] E. R. Squibb and Sons, Princeton, N.J.
[22] Raney nickel.

[23] PM-10 filter unit, Amicon Corp., Danvers, Mass..



Scheme I

pH 7.0, containing 0.02% bovine serum albumin. Antibody-bound and free radioligand were separated by addition of polyethylene glycol 6000 at a concentration of 130 g/liter in assay buffer. After incubating with the polyethylene glycol 6000 for 30 min at 0°, the immunoglobulins were pelleted by centrifugation (4000×*g*) for 30 min at 4° and the supernate, containing free radioligand, was decanted. For assays employing the tritium label, the immunoglobulin pellet was redissolved in assay buffer (0.3 ml), scintillation fluid was added (3 ml), and the vials capped, mixed, and placed in specially prepared vials for liquid scintillation counting. For assays employing the radioiodine label, the tubes containing the immunoglobulin pellets were placed into a gamma counter. All assay points were established in duplicate. Raw scintillation counter data were automatically entered and stored in a computer file for later processing by computer program. All meobentine concentrations determined by radioimmunoassay are expressed as meobentine-free base, while doses administered are expressed as meobentine sulfate.



Scheme II

Antiserum Titrations—Varying dilutions of antiserum in assay buffer (0.5 ml) and normal blank plasma (0.1 ml; dog, rat, or human) were incubated with [^{3}H]meobentine (2 ng, 12,000 dpm) in buffer (0.5 ml) or [^{125}I]iodomeobentine (0.25 ng, 40,000 dpm) in buffer (0.5 ml) overnight at 4°. Background-binding tubes contained only radioligand and buffer. Aliquots of polyethylene glycol 6000 solution in assay buffer (1.1 ml) were added to the ice-cold incubation tubes, and the contents were vortex-mixed, incubated at 0° for 30 min, and centrifuged (4000×*g*, 30 min). After decanting the supernates, the immunoglobulin pellets were prepared for either liquid scintillation or gamma counting as described previously. The antiserum titer chosen for use in the radioimmunoassay was defined as that dilution which bound 40% of the total radioligand added.

Antisera Specificity—To determine the specificity of the antisera,

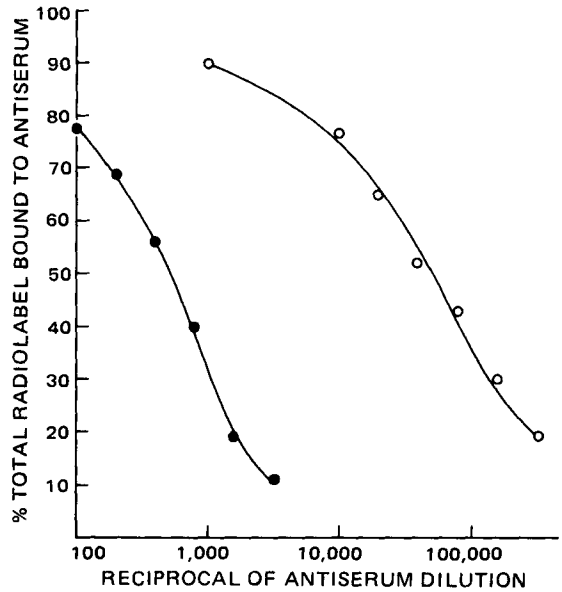


Figure 1—*Titration curves for meobentine antiserum used in the present experiments. Key:* ●, *[^{3}H]meobentine;* ○, *[^{125}I]iodomeobentine.*

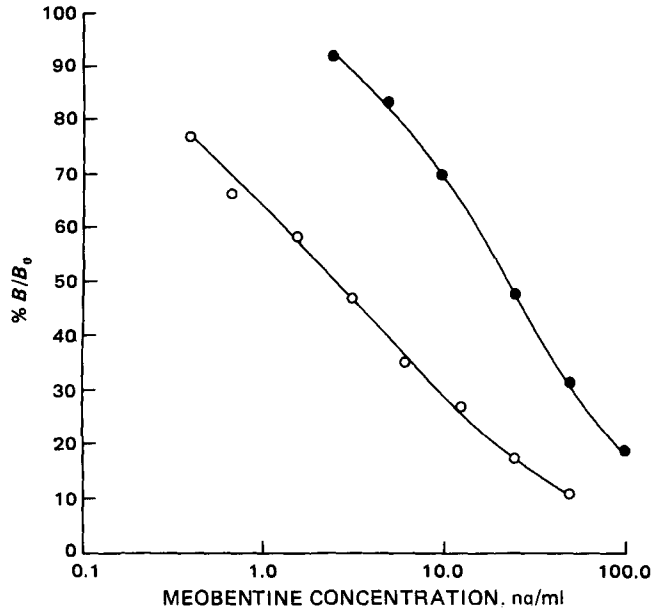


Figure 2—*Meobentine standard curves for tritium and radioiodine radioimmunoassays: mean of 8 curves in each case. Key: ●, [^{3}H]meobentine; ○, [^{125}I]iodomeobentine.*

the 0.1 ml of normal blank plasma added in the previously described procedure was replaced by 0.1 ml of increasing concentrations of meobentine, known metabolites, structurally related compounds, or various cardiovascular drugs in normal blank plasma. Incubation with radioligand and predetermined titer of antisera in assay buffer followed by isolation of the antibody-bound radioligand proceeded as described previously. Standard curves were expressed as percent B/B_0 *versus* log meobentine free base concentration, where B_0 represents the amount (cpm) of labeled meobentine (^{3}H or ^{125}I) bound in the absence of any unlabeled meobentine, and B is the amount bound in the presence of a given drug concentration (corrected for nonspecific binding). The cross-reactivity of each related compound was defined as the percentage ratio of the IC_{50} for meobentine to that of the compound studied, where IC_{50} is the concentration of each respective compound required to inhibit binding of radioligand to antiserum by 50% (10).

Radioimmunoassay of Unknown Plasma Samples—A series of meobentine standard solutions (2.5–250 ng/ml free base equivalents for ^{3}H-radioimmunoassay or 0.39–100 ng/ml for ^{125}I-radioimmunoassay) in normal blank plasma (0.1 ml) was incubated with [^{3}H]meobentine or [^{125}I]iodomeobentine and appropriately diluted antiserum as described previously. Spiked control samples of meobentine in blank plasma (0.1 ml) or unknown plasma samples appropriately diluted with normal blank plasma to enter the assay range were also incubated with radioligand and antiserum. Most unknown samples were assayed at two dilutions. Standard curves were linearized by a logit–log computer transformation of B/B_0 *versus* meobentine concentration data and unknown concentrations were determined by interpolation.

RESULTS

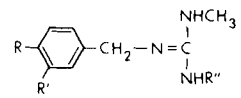
Immunogen Synthesis—A propionic acid analog (VI) of meobentine, with the acid chain attached to the phenyl ring *ortho* to the methoxyl group, was prepared from 3-(5-bromo-2-methoxyphenyl)propionic acid (I) by displacing bromine with cuprous cyanide to give the 5-cyano derivative (II) which was catalytically reduced to the benzylamine (III). The general method of Maxwell and Walton (8) was employed to convert the benzylamine derivative to IV, *via* the intermediates, IV and V. The synthetic scheme is shown in Scheme I. Elemental and spectral analysis established the identity of the hapten, which was coupled to bovine serum albumin by the mixed anhydride approach, described previously (11). No attempt to quantitate the extent of hapten incorporation to the immunogen was made.

Table I—Interassay Accuracy and Precision of Meobentine Radioimmunoassay Procedures

	Tritium Radioimmunoassay			
Spiked concentration[a]	3.0	15.0	30.0	150.0
Mean measured concentration	2.7	14.6	28.8	142.8
Coefficient of variation (n = 14)	11.1	5.5	5.2	6.0
	Radioiodine Radioimmunoassay			
Spiked concentration[a]	0.90	3.0	30.0	
Mean measured concentration	0.86	2.9	33.2	
Coefficient of variation (n = 15)	5.8	8.6	7.5	

[a] Concentration of meobentine sulfate (expressed as ng/ml free base equivalents) added to blank human plasma.

Table II—Cross-Reactivities of Related Compounds in Meobentine Radioimmunoassay Procedures



Compound	% Cross-Reactivity: Tritium Assay	% Cross-Reactivity: Radioiodine Assay
Meobentine (R = OCH_3, R′ = H, R″ = CH_3)	100 (IC_{50} = 22 ng/ml)	100 (IC_{50} = 2.2 ng/ml)
Hapten derivative (VI; R = OCH_3, R′ = $(CH_2)_2CO_2H$, R″ = CH_3)	116	340
O-Desmethylmeobentine (R = OH, R′ = H, R″ = CH_3)	4.7	2.3
N-Desmethylmeobentine (R = OCH_3, R′ = R″ = H)	2.4	3.4
Bethanidine (R = R′ = H, R″ = CH_3)	4.7	4.7
4-Methylbethanidine (R = R″ = CH_3, R′ = H)	NT[a]	41.0

Guanethidine, guanabenz, clonidine, bretylium, guanidine, 1,1-dimethylguanidine, *N*,*N*′-dimethylurea, *N*,*N*′-dimethylthiourea, procaine, procainamide, lidocaine, propranolol, isoproterenol, and morphine all showed <0.001% cross-reactivity.

NT—not tested.

Radioligand Syntheses—[^{3}*H*]*Meobentine*—This compound was obtained from meobentine by catalytic exchange of tritium for hydrogen with subsequent removal of labile tritium. The resulting, generally tritiated radioligand had a relatively low specific activity (0.8 Ci/m*M*). This feature limits the usefulness of the radioimmunoassay employing [^{3}H]-meobentine due to low sensitivity and lengthy scintillation counter times.

[^{125}*I*]*Iodomeobentine* (*IX*)—The need for a radioligand with much higher specific activity to enable the development of a more efficient and sensitive radioimmunoassay for meobentine necessitated the synthesis of a meobentine analog containing iodine 125 (Scheme II). An analog (and

Table III—Correlation of Plasma Meobentine Concentrations Determined by ^{3}H- and ^{125}I-Radioimmunoassays[a]

Sample	Meobentine Concentration (ng/ml): ^{3}H-Radioimmunoassay	^{125}I-Radioimmunoassay
Human	255	264
	514	485
	524	390
	512	530
	638	613
	254	238
	294	281
	411	370
	516	556
	475	495
Dog	9909	9341
	2772	2559
	1299	1215
	873	838
	618	538
	273	249
	353	315
	279	274
	256	264
	195	204
Rat	7837	7335
	3335	3478
	6799	6568
	3583	3470
	4097	3900

[a] Linear Regression Analysis Parameters: correlation coefficient = 0.999, slope = 0.949, y-intercept (^{3}H-radioimmunoassay) = 11.6 ng/ml.

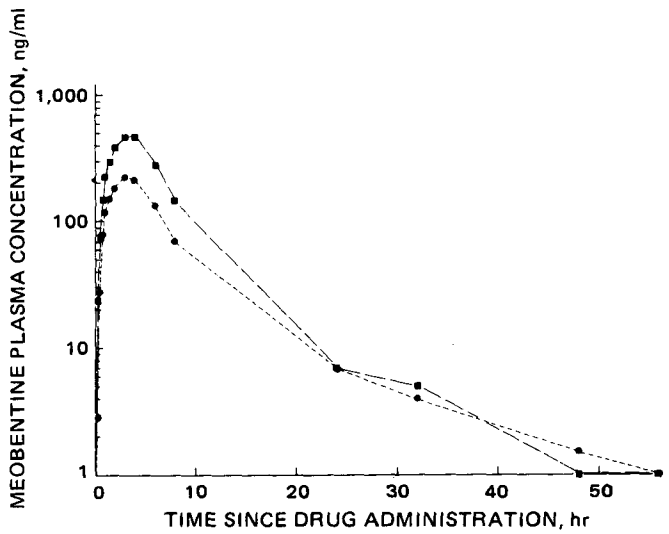


Figure 3—*Mean plasma meobentine concentrations (expressed as free base) following oral administration to normal human volunteers. Key: ●, 2.5 mg/kg (n = 6); ■, 5.0 mg/kg (n = 5).*

metabolite) of meobentine, VII, was synthesized from 4-hydroxybenzylamine (8). The phenol (VII) was *ortho*-iodinated with [^{125}I]sodium iodide by the method of Hunter and Greenwood (12). *In situ* methylation of the iodophenol with diazomethane followed by TLC purification afforded iodomeobentine of high specific activity.

Antisera Production—Two out of three rabbits immunized with the hapten produced useful antisera, detectable within 1 month and steadily increasing in titer with time. The highest titer elicited from the best rabbit was selected for further studies. Excess antibody bound > 90% of either radioligand and the working titers were 1/4000 and 1/100,000 for [^{3}H]-meobentine and [^{125}I]iodomeobentine, respectively (Fig. 1).

Assay Sensitivity and Precision—The sensitivity limit of the radioimmunoassay for meobentine sulfate employing a tritium or radioiodine label (defined as the concentration of unlabeled meobentine producing 10% inhibition of maximal [^{3}H]meobentine or [^{125}I]iodomeobentine binding) was 2.5 or 0.39 ng/ml, respectively. Typical mean standard curves for the two assay procedures are shown in Fig. 2; the coefficient of variation around each standard concentration point was <10%. Further confirmation of interassay precision was provided by the low coefficient of variation around means of assayed spiked plasma controls varying across the range of assay sensitivity (Table I).

Assay Specificity—Specificity data of this antiserum for meobentine, some known metabolites, and structurally related compounds are summarized in Table II for both tritiated and radioiodinated meobentine radioligands. Cross-reactivities with the two known metabolites, *O*-desmethyl and *N*-desmethylmeobentine, were low, being 2–5% in both assays. The desmethoxy analog, bethanidine, also showed relatively low cross-reactivity (5%) while the *para* methyl derivative, *N*-4-methylbenzyl-*N'*,*N''*-dimethylguanidine, was recognized much better by the antiserum, resulting in a higher (40%) cross-reactivity. As expected, the propionic acid hapten derivative (VI) used for the production of the immunizing conjugate was recognized by the antiserum more effectively than meobentine itself. A variety of other structurally related guanidines with (guanethidine, guanabenz, and clonidine) and without (guanidine and dimethylguanidine) substituents on the imino nitrogen atom showed little cross-reactivity. A number of drugs (procaine, procainamide, lidocaine, propranolol, isoproterenol, and morphine), which might be administered to patients concurrently with meobentine sulfate, had cross-reactivities of <0.001% in the radioimmunoassay.

Correlation of Tritiated and Radioiodinated Assay Methods—Since both assay methods were used in the analysis of different phases of the human disposition studies, it was imperative to demonstrate that analysis of given samples by both methods gave the same results. To this end, a number of plasma samples obtained from humans, dogs, and rats after meobentine treatment were analyzed by both methods. The results are shown in Table III. The slope and linear regression coefficient approach unity, indicating good agreement between results from tritiated and radioiodinated methods.

Disposition Studies in Humans—The radioimmunoassay employing [^{3}H]meobentine was used to assay plasma samples from humans who received two separate oral doses (2.5 and 5 mg/kg) of meobentine sulfate in water. Mean plasma levels of meobentine in these subjects are shown in Fig. 3. No detailed pharmacokinetic analysis was performed on these data, but some model-independent mean kinetic parameters are collated in Table IV. Mean observed maximum plasma concentrations were 239 and 451 ng/ml following 2.5 and 5.0-mg/kg doses, respectively (Table IV). The mean area under the plasma concentration–time curve (*AUC*) values increased ~1.8-fold on increasing the dose from 2.5 to 5 mg/kg. Thus, similar trends are evident in C_{max} and *AUC* values. The mean times to observed peak plasma concentration (T_{max}) were 3.3 and 3 hr after each of the 2.5 and 5-mg/kg doses, respectively. Meobentine half-life, as estimated from the limited number of points in the terminal disposition phase, ranged from 9 to 20 hr between individual subjects, while mean values following treatments were ~ 14 and 11 hr.

Table IV—Model-Independent Pharmacokinetic Parameters for Meobentine Sulfate (Mean ± *SD*)

Parameter	Oral Dose Administered	
	2.5 mg/kg (n = 6)	5.0 mg/kg (n = 5)
C_{max}, ng/ml	239.0 ± 68.4	451.0 ± 173.9
T_{max}, hr	3.3 ± 0.5	3.0 ± 0.7
AUC, hr ng/ml	1890 ± 526	3358 ± 1294
TBC/F, liter/hr[a]	78.6 ± 14.9	95.1 ± 29.6

[a] Total body clearance/fraction dose absorbed = dose/area under the curve (*AUC*).

DISCUSSION

Previous investigations have demonstrated the need for conjugation of drugs to macromolecules in order to render them immunogenic (5, 6, 13). In the present study, meobentine was coupled *via* a propionic acid sidechain to bovine serum albumin by a mixed anhydride procedure. Attachment of the 3-carbon bridge was *ortho* to the methoxyl group of meobentine, as shown in Scheme I. Antibodies formed in response to repeated immunization with this substance were used to develop two radioimmunoassay procedures for meobentine. One assay, employing a generally labeled [^{3}H]meobentine ligand, was precise and accurate and showed a sensitivity limit of 2.5 ng/ml (250 pg, actual mass). The use of [^{125}I]iodomeobentine (Scheme II), which had much greater specific activity, considerably increased efficiency and lowered sensitivity limits to <0.4 ng/ml (40 pg, actual mass) while retaining precision and accuracy (Fig. 2 and Table I). Plasma meobentine levels in the rat, dog, and humans following intravenous and oral administration, assayed by the two different procedures, agreed well (Table III). These radioimmunoassay procedures represent a significant advance in sensitivity and convenience over previously available methods for determination of meobentine in plasma. The sensitivity of the procedure using the iodinated radioligand, which is currently in use for analysis of meobentine disposition studies, exceeds that of analytical procedures reported for the related drugs, bethanidine (14) and bretylium (15, 16).

The ability of drug antisera to discern changes in molecular architecture normally is greatest for structural changes at sites distant from the site of attachment of the drug to macromolecular carrier in the immunizing conjugate. The applicability of this statement to the case of meobentine is not complete, as the data in Table II indicate. Thus, 4-methylbethanidine cross-reacts extensively, as expected for a relatively minor structural change close to the site of attachment of hapten to protein. However, the cross-reactivities of bethanidine (4.7%) and *O*-desmethylmeobentine (2.3–4.7%), which also involve relatively minor structural changes at the *para* position, are much lower. It is fortunate that the cross-reactivity of *O*-desmethylmeobentine is low, since this compound appears to be a major metabolite in the rat and dog (17), although the most likely circulating species, the *O*-glucuronide of *O*-desmethylmeobentine, would be expected to cross-react even less. *N*-Desmethylmeobentine, a lesser metabolite in the rat and dog (17), has lower cross-reactivity. The *O*-desmethoxy analog, bethanidine, while undergoing significant *N*-dealkylation in the rat and dog, has been reported to be excreted largely unchanged by humans (18). Bethanidine is not well recognized by the antiserum, while the 4-methyl analog binds strongly to the antibodies, indicating the sensitivity to changes in molecular charge localization and lipophilicity. A wide range of structurally similar guanidines and other cardiovascular drugs and potentially concurrently administered medications did not cross-react.

The applicability of the present radioimmunoassay techniques to pharmacokinetic studies is shown by the data in Fig. 3. Plasma levels 56 hr after a single oral dose could still be measured reliably by the [^{3}H]-meobentine assay, which was used for analysis of plasma samples in the

human studies described in this paper. The higher sensitivity and greater convenience of the [^{125}I]-based assay have resulted in its use in current meobentine studies. The plasma profile in the subjects studied indicated that meobentine kinetics are multicompartmental in nature with a long terminal half-life (11–14 hr). A long terminal half-life has also been seen with bethanidine (18) and bretylium (19) in humans. Mean peak meobentine plasma levels in the same group of volunteers essentially doubled from 239 to 451 ng/ml with an increase in dose from 2.5 to 5.0 mg/kg. Mean area under the curve also approximately doubled from 1890 to 3358 ng hr/ml over this dose range. These observations suggest linear kinetics for meobentine in the 2.5–5-mg/kg dose range. Clearance (TBC/F) did not change significantly with increasing dose. The extent of absorption and bioavailability of meobentine are currently under investigation in animals and humans. The related guanidine, bethanidine, has been reported to be well absorbed by humans (18).

Currently, these radioimmunoassay procedures are being applied to detailed studies of the disposition of meobentine sulfate in animals and humans. Studies of the absolute oral bioavailability and pharmacokinetics of meobentine sulfate will be presented elsewhere in the near future.

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Automated Sampling of *In Vitro* Dissolution Medium: Effect of Sampling Probes on Dissolution Rate of Prednisone Tablets

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Abstract □ The effect of sampling probe size and location on the *in vitro* dissolution rate of prednisone tablets was examined. Using USP XX Apparatus 2 with an automated sampling system, dissolution rates were determined using two types of large filter-tipped probes and a small capillary probe. Each probe was tested at three locations within the kettle. The large probes caused hydrodynamic changes which, when compared with results obtained through manual sampling, resulted in significant changes in dissolution rates at each location. These changes were less evident when the capillary probe was used, with an insignificant difference between results of automated and manual sampling when the capillary probe was placed midway between the paddle shaft and the kettle wall.

Keyphrases □ Dissolution rates—effect of sampling probe size and location on dissolution rate of prednisone tablets □ Hydrodynamics—dissolution rates affected by sampling probe size and location, USP paddle method □ Prednisone—tablets in dissolution rate testing with effects of sampling probe size

Automated sampling and analysis of *in vitro* dissolution aliquots can be a useful, timesaving procedure. Several automated sampling systems are commercially available in which aliquots of the dissolution fluid, taken at specified times, travel into sample cups or into an automated analytical system. Results of such a system are not generally considered acceptable, however, unless they agree with those obtained by manual sampling.

A large number of dissolution testing variables have been identified (1). These need to be strictly controlled if reproducible results are to be obtained. Changes in any of these variables can have a significant effect on the dissolution rate of a dosage form. A previous study (2) showed that the dissolution rate is affected by the effects of various dissolution parameters on the hydrodynamics of the dissolution fluid. Another study (3) showed that the hydrodynamic disturbance caused by insertion of a relatively large object, such as a thermometer, into the dissolution fluid may cause a change in dissolution rate, and that the location of such an object can also have a significant effect.

With most automated sampling systems now in use, a relatively large filter-tipped probe is immersed in the dissolution fluid. The above studies imply that a large probe can affect the system hydrodynamics, and therefore the dissolution rate of some dosage forms, thereby causing results which differ from those obtained by manual sampling, and that a smaller probe may have less effect on the

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