The degree of protonation (α) of the reaction can be written as:

$$\alpha = \frac{[I^-]}{[I + I^-]} = \frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 3)}$$

The overall rate constant k_{dep} consists of:

$$k_{dep} = k_t^{I}[I] + k_t^{I^-}[I^-] = k_t^{I}(1 - \alpha) + k_t^{I^-}(\alpha) \quad \text{(Eq. 4)}$$

where k_t^{I} is the rate constant for tautomerization of uncharged I and $k_t^{I^-}$ the same constant for I^- (deprotonated I). Combination of Eqs. 3 and 4 yields:

$$k_{dep} = (k_t^{I}) \frac{[H^+]}{K_a + [H^+]} + (k_t^{I^-}) \frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 5)}$$

Assuming that, with respect to tautomerization, I is much more active than I^- or that $k_t^{I} \gg k_t^{I^-}$, it is obvious that on increasing the pH, the term $k_t^{I}([H^+]/K_a + [H^+])$ will decrease more rapidly than the term $k_t^{I^-}([H^+]/K_a + [H^+])$ increases which will result in a lower value of k_{dep}. Under the assumption that $k_t^{I^-}$ is so small that the term $k_t^{I^-}(K_a/K_a + [H^+])$ can be neglected, Eq. 5 permits the estimation of k_t^{I}, using pK_a 12.44 of the acidic group and the values for k_{dep} listed in Table I. The calculation results in a k_t^{I} of $1.4 \pm 0.4 \times 10^{-1}$ sec^{-1} and a half-life ($t_{1/2}$) of the process of 5.0 sec. The explanation of the concentration dependence of k_{dep} at pH < pK_a lies in the aforementioned possibility of interaction between the 7-amino–quinoid moieties on increasing [I]. The occurrence of these interactions is shown by the changes in absorptivity at higher [I] at pH 10, where the compound is present almost completely in the uncharged form. The decrease in the molar absorptivity must be due to interaction of the 7-amino–quinoid chromophores. Since the tautomerization occurs in the same functional group in the molecule, this interaction most likely also causes a decrease in the overall rate constant k_{dep} at higher [I] at pH 12.0, as the concentration of the unaffected 7-amino–quinoidal function decreases due to this interaction. Equation 4 shows that on decreasing [I] and $[I^-]$, k_{dep} will decrease. At pH 13.0, where $[I] = 0.2\ [I + I^-]$, the concentration of uncharged I in the range studied is too low for aggregation to occur.

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NOTES

Tissue Distribution of [^{14}C]Bretylium Tosylate in Rats

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Received March 2, 1982, from the *Department of Pharmaceutical Development, Research and Development Department, American Critical Care, McGaw Park, IL 60085.* Accepted for publication April 21, 1982. *Present address: Xavier University, New Orleans, LA 70125.

Abstract ☐ The distribution of [^{14}C]bretylium tosylate in the body and the relationship between tissue and plasma concentrations was determined following intravenous administration of the drug to Charles River rats. The renal excretion of bretylium was rapid in rats and follows an active process. On the average, 50% of the administered dose was excreted in the urine within 1 hr. In the postequilibrium phase, the plasma concentration declined with a half-life of 5 hr. Bretylium concentrations in all tissues, except the heart, declined rapidly according to a triexponential equation. The liver and kidney bretylium concentrations declined in parallel to the plasma concentration with mean tissue–plasma concentration ratios of 6.04 and 12.3, respectively, in the β phase. However, the concentration of bretylium in the heart increased gradually and peaked at 2 hr, with a tissue–plasma concentration ratio of 121, which, in turn, declined to a value of >60 after 8 hr. The data indicated that (*a*) bretylium is rapidly distributed into the liver and kidney immediately after reaching the systemic circulation; (*b*) the distribution into the heart occurs at a slower rate compared with the other organs, and the drug has a high affinity to the myocardium; and (*c*) since the heart is the site of action and there is no direct correlation between the concentrations in myocardium and plasma, the antiarrhythmic effect of bretylium may not be related to the plasma concentration.

Keyphrases ☐ Bretylium tosylate—^{14}C-labeled, tissue distribution, rats, plasma, renal excretion ☐ Distribution—tissue, [^{14}C]bretylium tosylate in the rat, plasma, renal excretion ☐ Excretion, renal—tissue distribution of [^{14}C]bretylium tosylate in rats, plasma

Bretylium tosylate is a quaternary ammonium salt given in the treatment of cardiac arrhythmia. It suppresses *ventricular* fibrillation within minutes of intravenous infusion (1). The suppression of ventricular tachycardia, however, developed more slowly, usually 20–120 min after intramuscular administration of 4 mg/kg of bretylium tosylate in humans (2). The effect of bretylium in the intact heart was shown to be biphasic, with an initial transient increase in blood pressure and heart rate followed by an opposite and more prolonged depression of these same

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Table I—Tissue to Plasma Concentration Ratios of [^{14}C]Bretylium Tosylate During 8 hr After Drug Administration

Hours	Tissue–Plasma Concentration Ratio						
	Serum	Red Blood Cells[a]	Liver	Kidney	Heart	Lung	Spleen
0.1[b]	1.13	1.23	14.1	25.8	3.23	3.23	3.19
0.5	1.30	1.41	19.2	27.0	16.1	—	—
1.0[b]	1.69	1.82	16.2	19.3	57.9	16.4	6.86
2.0	1.80	2.05	11.6	17.3	121	—	—
4.0[b]	2.19	2.56	6.69	14.6	94.1	9.78	11.6
6.0[b]	1.86	1.89	4.69	9.48	60.0	7.93	8.07
8.0	2.16	2.48	6.74	12.8	69.5	—	—
Mean[c] β-Phase Ratio	2.07	2.31	6.04	12.3	74.5	8.86	9.84

[a] Amount of drug in red blood cells was determined by amount in whole blood minus amount in plasma, where amount in plasma was determined by plasma concentration [volume of whole blood (1-hematocrit)] and red blood cell concentration was determined by amount/(volume of whole blood × hematocrit). [b] Average ratios from eight rats for red blood cells, liver, kidney, and heart. Lung and spleen values and all other values are average ratios from four animals. [c] The β-phase ratio was calculated from the linear portion of each curve, *i.e.*, 4–8 hr for each tissue.

parameters (3). These effects probably can be related to the time course of bretylium in the heart following administration.

In dogs, the myocardial drug concentrations were shown to increase gradually with a peak occurring at 1.5–6.0 hr after intravenous administrations of 2 and 6 mg/kg of bretylium tosylate (4). The myocardial tissue–serum concentration ratio increased progressively to 6.4–12.6 after 12 hr. In the same study, the antifibrillatory effect was correlated with the myocardial levels of bretylium but not with serum concentrations.

Pharmacokinetic studies in humans and animals showed that bretylium has a very large volume of distribution (5–7), indicating a high affinity to tissues. Over 80% of a 5-mg/kg bretylium tosylate dose is excreted unchanged in urine by active renal excretion processes (5, 8). The average biologic half-life of bretylium is 8 hr in four normal subjects (5) and becomes longer in patients with impaired renal function (9).

In rats, the biologic half-life of bretylium is ~5 hr (7). An average of 94–95% of a 10-mg/kg [^{14}C]bretylium tosylate dose was recovered in 72 hr, of which 63–65% was excreted unchanged in urine and 30–31% was eliminated unchanged in feces following intravenous administration, indicating a pronounced biliary excretion (7, 8). The purpose of the present investigation was to examine the distribution pattern of bretylium in well-perfused organs, heart, kidney, and liver, which are the sites of pharmacological action, urinary excretion, and possible metabolism of the drug, respectively, in rats.

EXPERIMENTAL

This investigation was carried out in two separate studies and on two different occasions. For this purpose, male Charles River rats[1] weighing 300–400 g were used. Custom synthesized [^{14}C]bretylium tosylate labeled on the benzylic carbons (specific activity 27.3 μCi/mg, 99% purity) was purchased[2]. The labeled compound was mixed with cold bretylium tosylate[3] and dissolved in saline to produce the injection solution (5 mg/ml, specific activity 3.4 μCi/mg).

In study I, 28 animals were divided into seven groups of four rats each. Each animal received an intravenous injection of 10 mg/kg of [^{14}C]-bretylium tosylate, and each group was then sacrificed by portal vein bleeding under ether anesthesia at the following times postdose: 0.1, 0.5, 1, 2, 4, 6, and 8 hr. A portion of the blood sample was centrifuged in a tube containing dry heparin to separate plasma, and the other was allowed to coagulate and then centrifuged to collect serum. An aliquot of the heparinized blood was dispensed into a combustion cup for total radioactivity determination in whole blood. The kidneys, liver, and heart were removed.

Study II was performed approximately 1 year later. Sixteen animals were divided into four groups of four rats each. Each animal received an intravenous dose of 10 mg/kg of [^{14}C]bretylium tosylate, and each group was sacrificed at 0.1, 1, 4, and 6 hr after drug administration, in the same manner as described in study I. Again plasma and serum were collected. In addition, the kidneys, liver, heart, lung, spleen, and carcass were separated. All urine and feces were quantitatively collected.

Immediately after removal, the organs were sliced, blotted in tissue paper to remove excess blood, and weighed. To the organs, the carcass, and the feces, a saline solution 3–5 times the weight of the organ was added. The mixture was then homogenized[4] in an ice bath. An aliquot of each homogenate was frozen and stored until assay.

The total radioactivity in plasma was determined on an aliquot of the samples in a liquid scintillation counter[5]. The total radioactivity in blood, tissue, and feces samples was measured after combustion[6] of an aliquot of the homogenate and by counting the processed samples in the liquid scintillation counter.

Mean concentrations (n = 4) in the plasma, serum, red blood cells, liver, and kidney were fitted to a triexponential equation, describing a typical three-compartment open model (7). The concentration in the heart did not follow the same model. The terminal disposition half-life in myocardium was calculated using nonlinear regression analysis of a concentration–time profile. The data from study II served for comparative purposes and allowed the estimation of the percentage of the dose remaining in various tissues at different times.

RESULTS AND DISCUSSION

Figure 1 depicts the time course of bretylium concentration in various tissues and plasma following intravenous administration. The data from study II, which was conducted 1 year later, were included. There was good agreement between the two sets of data showing an *excellent reproducibility* of bretylium pharmacokinetics in rats. The concentrations in plasma, liver, and kidney declined in parallel following intravenous administration. In general, there was a good fit of these data to a triexponential equation describing a three-compartment open model. The elimination half-life was 5 hr, which is in agreement with the previously reported value (7).

The accumulation and decline of bretylium concentration in the heart resembled the simulated time course of the drug in a deep peripheral compartment. There was a gradual accumulation of bretylium in the heart with a peak occurring between 1–2 hr. This was also in agreement with reported data of a gradual increase in myocardium levels in dogs (4). The postdistribution decline in bretylium heart concentration, however, was more rapid, showing a biological half-life of 2 hr. It is possible that this decline is part of a redistribution process and that concentration in the heart will eventually reach an equilibrium with that in plasma and other tissues. Since the heart is the site of action, it will be expected that antiarrhythmic action will be associated with the concentration of bretylium in myocardium. Such a situation has been demonstrated in

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] New England Nuclear, Boston, Mass.
[3] American Critical Care, McGaw Park, Ill.
[4] Polytron Homogenizer, Brinkman Instruments, Inc., Westbury, N.Y.
[5] Models 425 and 460C, Packard Instruments, Inc., Downers Grove, Ill.
[6] Sample Oxidizer Model B306, Packard Instruments, Inc., Downers Grove, Ill.

Table II—Percent of Dose Excreted in Urine and Feces and Remaining in Selected Organs Following Intravenous Administration of Bretylium Tosylate to Rats

	Percent of Dose[a]							
Hours	Blood	Kidney	Liver	Heart	Spleen	Lung	Urine	Feces
0.1	4.49	12.0	29.6	0.972	0.364	0.786	—	—
	(0.327)	(3.68)	(1.87)	(0.029)	(0.053)	(0.084)		
1.0	0.916	1.14	5.69	1.43	0.156	0.653	49.2	1.29
	(0.120)	(0.187)	(1.04)	(0.104)	(0.006)	(0.042)	(3.30)	(1.25)
4.0	0.339	0.375	0.694	0.579	0.069	0.099	55.9	0.733
	(0.011)	(0.208)	(0.123)	(0.209)	(0.004)	(0.020)	(6.12)	(1.465)
6.0	0.215	0.143	0.388	0.288	0.038	0.061	61.1	3.48
	(0.066)	(0.016)	(0.097)	(0.101)	(0.015)	(0.010)	(1.66)	(1.04)

[a] Mean of four rats, standard deviation in parentheses.

dogs (4). Thus, until an equilibrium between myocardium and plasma levels is attained, it is reasonable to presume that there will be no correlation between antiarrhythmic action and plasma concentration.

Table I summarizes the tissue-plasma concentration ratios of serum, red blood cells, liver, kidney, heart, lung, and spleen tissues. Table II summarizes the tissue content of bretylium and amounts excreted in urine and feces at various times after intravenous administration of the drug. For both liver and kidney, which are well-perfused organs, the tissue-plasma concentration ratios were generally higher in the distribution phase than in the β-phase during which average equilibrium ratios of 6.0 and 12.3, respectively, were attained. At 0.1 hr, the uptake of bretylium in liver and kidney accounted for 30 and 12% of the dose, respectively. The high bretylium levels in the liver and kidney are consistent with rapid elimination of the drug: ~50% of the dose is excreted in urine within 1 hr (Table II). Also, it was demonstrated that about one-third of the dose is eliminated by biliary excretion (7).

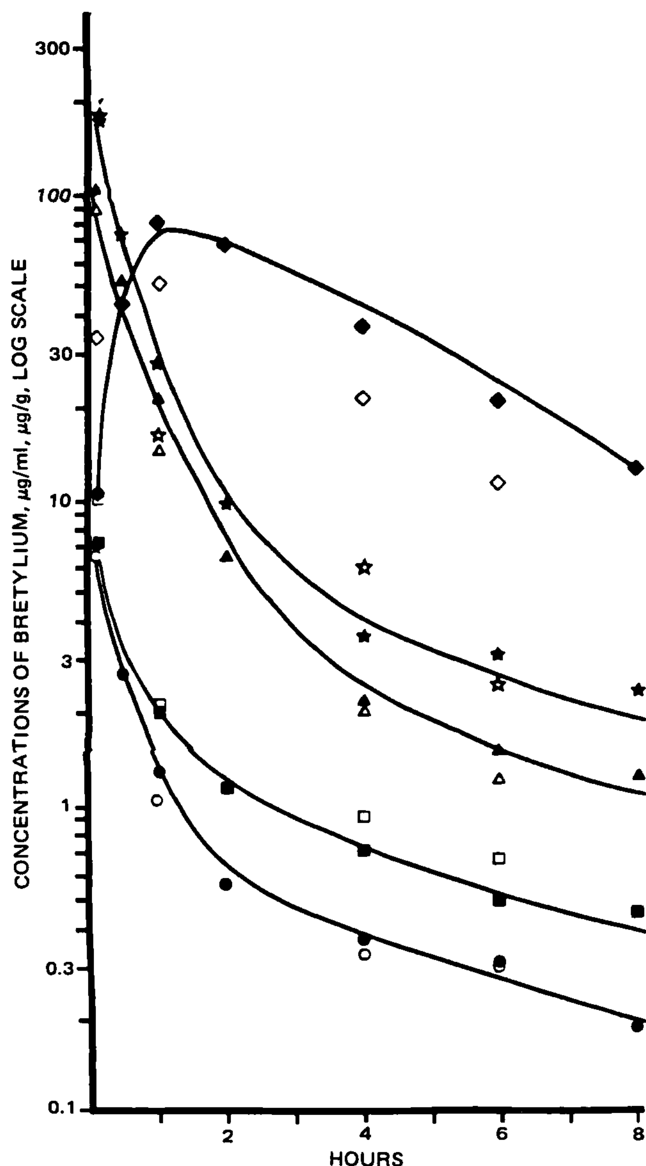

Figure 1—*Mean concentration of bretylium in plasma (●), red blood cells (■), liver (▲), kidney (★), and heart (◆) following intravenous administration of 10 mg/kg of bretylium tosylate in rats in study I. The respective open symbols represent data from study II. Observations from study I were used to fit the curves. Each data point is the mean of four animals. The concentrations in plasma are given by micrograms per milliliter and in the tissues by micrograms per gram.*

The heart-plasma concentration ratio increased gradually from 3.2 after 6 min to 121 after 2 hr. The peak ratio was much higher than that observed in dogs, 12.6 (4), which may indicate species differences in bretylium distribution in the body. There was a sharp decrease in the heart-plasma concentration ratio to ~70 after 8 hr. The continuous change in this concentration ratio is consistent with delayed onset of action followed by a more prolonged effect reported in the literature (3, 4).

For red blood cells, there was also a gradual increase in the tissue-plasma concentration ratios, reaching an apparent equilibrium within 2 hr. This gradual change in the ratios may be due to either a rapid distribution in the cells followed by a slower rate of redistribution in the plasma or a gradual uptake of the drug by red blood cells against a concentration gradient, presumably by an active mechanism. The lung and spleen uptake also appears to be similar to that in the red blood cells. An active tissue uptake has been suggested for another quaternary ammonium compound, tetraethylammonium, in rats (10).

One hr after drug administration, on the average, 49 and 1.3% of the dose were found in the urine and feces, respectively. The initial rapid elimination of bretylium in urine was proportional to the initial high plasma concentration (8 μg/ml). When the plasma level declined to <1 μg/ml (1 hr postdose), the urinary excretion accounted for ~12% over the next 5 hr. Of the remaining 50% at 1 hr, 10% of the dose could be accounted for in blood, kidney, liver, heart, spleen, and lung tissues. This amount decreased significantly to ~2% at 6 hr following administration.

The data suggest that at 1 hr, 39% of the dose should be accounted for in the carcass. The overall radioactivity determined in the carcass, however, was equivalent to 50% of the dose. Unfortunately, the homogenization of the carcass was later found to be incomplete, and it is possible that this caused the overestimation of body content by nearly 10%. Previously, it was shown that about one-third of the bretylium dose was eliminated in the feces through biliary excretion (7). Therefore, the intestines might contain most of the radioactivity which was found in the carcass.

In summary, bretylium is rapidly distributed in the body, particularly in the liver and kidneys, immediately after reaching systemic circulation. While there was a parallel decline in liver, kidney, and plasma concentrations of bretylium, there was no correlation between plasma and heart concentrations of the drug. The heart showed high affinity to bretylium with its concentration reaching 120 times plasma levels within 2 hr. These data suggest that the antiarrhythmic effect of bretylium will not be associated with its plasma concentrations until an equilibrium between heart and plasma concentrations is attained.

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Room Temperature Phosphorescence Determination of Propranolol in Pharmaceutical Formulations

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Received March 19, 1982, from the *University of Florida, Department of Chemistry, Gainesville, FL 32611.* Accepted for publication May 14, 1982.

Abstract ☐ A simple, rapid, and specific procedure was used for the analysis of propranolol in pharmaceutical formulations. The procedure consisted of dissolving (diluting) appropriate quantities of preparations and standards in (with) a 2 *M* potassium iodide–ethanol–water solution, spotting 5 μl of each resultant solution onto filter paper disks, determining the phosphorescence intensities at room temperature, and comparing sample signal levels with those of standards. The results indicated that room temperature phosphorescence can be easily applied to the analysis of pharmaceutical formulations where active ingredients are generally contained in a wide variety of matrices.

Keyphrases ☐ Propranolol—room temperature phosphorescence determination in tablets and injections ☐ Phosphorescence—determination of propranolol in tablets and injections ☐ β-adrenergic agents—propranolol, room temperature phosphorescence determination, tablets and injections

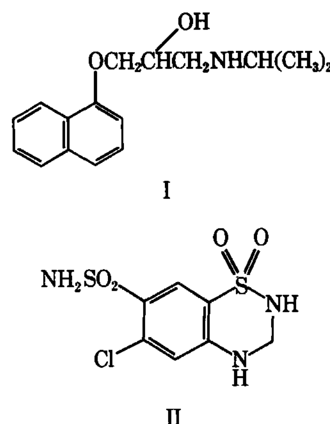
Propranolol hydrochloride, a β-adrenergic blocker, is commonly used for the treatment of nonacute hypertension, angina pectoris, and cardiac arrhythmias. With the patent on the only marketed product nearing its expiration, generic brands of propranolol will soon become available. Because generic manufacturers often use a variety of materials (diluents, binders, *etc.*) in their formulations, a specific procedure is needed for the quantitation of the active ingredient in the formulation.

The current USP procedure (1) for propranolol is cumbersome, and as a result, many pharmaceutical manufacturers are currently investigating fluorometry and high-performance liquid chromatography (HPLC) for quality control.

The present report describes a simple and rapid room temperature phosphorescence procedure applicable to the determination of propranolol in several pharmaceutical preparations. While room temperature phosphorescence has been shown to be analytically useful, real sample applications have been limited (2, 3). Overall, room temperature phosphorescence offers good selectivity with moderate sensitivity and detection power.

EXPERIMENTAL

Reagents—Propranolol hydrochloride[1] (I) and hydrochlorothiazide[2] (II) were used as received. All pharmaceutical preparations were purchased through a local hospital pharmacy[3]. All other materials were of analytical reagent grade.



Apparatus—All room temperature phosphorescence measurements were made with a spectrophotofluorometer[4] fitted with a 150-W xenon arc lamp[5], a laboratory-constructed phosphoroscope (4) for bar, room temperature phosphorescence (5), and a potted photomultiplier tube[6]. A ratio photometer[7] supplied high voltage to the photomultiplier tube in addition to serving as a DC amplifier. All line voltages were regulated with an AC regulator[8].

Standard and Sample Preparation—A standard stock solution (400 μg/ml) was prepared by dissolving an accurately weighed portion of propranolol hydrochloride in a 2 *M* potassium iodide solution (ethanol–water, 50:50). Standard solutions (5, 10, 20, 50, 100, 200, and 300 μg/ml) were prepared daily by mixing appropriate volumes of the stock solution with the ethanolic solution. Samples were prepared for assay by dissolution–dilution in/with the ethanolic solution. For analysis of representative samples, 20 tablets were weighed and powdered with a mortar and pestle, and four portions (equivalent to 100 μg/ml of active ingredient in a total volume of 10 ml) were dissolved in the ethanolic solution. This procedure was repeated for each solid sample. For the analysis of liquid preparations, appropriate volumes (same equivalence as tablets) of the samples were diluted with the ethanolic solution. Four different test solutions were prepared for each pharmaceutical formulation; each test solution contained 100 μg/ml of active ingredient.

Procedure—Bar, room temperature phosphorescence analysis consisted of an aluminum bar, a cover plate with four holes (each ~0.64-cm diameter) and filter paper disks[9]. The filter paper disks were placed under the cover plate and the cover plate was tightened into place on the bar with four screws. Samples were spotted onto the paper disks in 5-μl vol-

[1] Ayerst Laboratories, New York, N.Y.: Inderal, propranolol hydrochloride; Inderide, propranolol hydrochloride with hydrochlorothiazide.
[2] Ciba Pharmaceutical Co., Summit, N.J.
[3] Pharmacy Stores, Shands Teaching Hospital, Gainesville, Fla.
[4] Aminco-Bowman SPF, American Instrument Co., Jessup, Md.
[5] Canrad-Hanovia, Newark, N.J.
[6] 1P21, Hamamatsu, Middlesex, N.J.
[7] American Instrument Co.
[8] Sorenson 1001, Norwalk, Conn.
[9] Grade 903, Schleicher & Schuell, Keene, N.H.

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