

# Metronidazole Phosphate—A Water-Soluble Prodrug for Parenteral Solutions of Metronidazole

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**Abstract** □ To develop a parenteral solution of relatively water-insoluble metronidazole (2-methyl-5-nitro-1H-imidazole-1-ethanol), its phosphate ester was synthesized *via* two routes. One route utilized 2-cyanoethyl phosphate and the other utilized pyrophosphoryl tetrachloride. The first method used dicyclohexylcarbodiimide as a coupling agent and the cyanoethyl group was removed under mild alkaline conditions. The second method was a one-step procedure in which free acid of metronidazole phosphate was isolated as a crystalline solid. The solubility of metronidazole in various solvents was determined at 25°. From the pH-dependence of its aqueous solubility, the pKa of the conjugate acid of metronidazole was estimated to be 2.62, which agreed well with the pKa values of other nitroimidazoles. Metronidazole phosphate behaved as a zwitterionic compound in an acidic medium with a minimum solubility at pH 2.0. At pH 7, its solubility was ~50 times that of metronidazole. The phosphate ester was so soluble at pH higher than 7 that it was difficult to measure the solubility accurately. In human serum, the hydrolysis of metronidazole phosphate followed zero-order kinetics at an initial concentration of 0.25 mg/ml or higher, presumably due to enzyme saturation (0.035 mg/ml/hr at 37°). A reversed-phase HPLC procedure was adopted to monitor the appearance of metronidazole and the disappearance of metronidazole phosphate. Subcutaneous administration of metronidazole phosphate to rats produced a blood level of bioactivity comparable to that observed after administration of metronidazole.

**Keyphrases** □ Metronidazole phosphate—a water-soluble prodrug for parenteral solutions of metronidazole □ Prodrugs—water-soluble, metronidazole phosphate for parenteral solutions of metronidazole □ Solubility—metronidazole phosphate, water-soluble prodrug for metronidazole

Although metronidazole is associated with some controversy regarding its carcinogenicity in rodents and mutagenicity in bacteria (1–6), it is often the drug of choice for the treatment of certain anaerobic infections, particularly *Trichomonas vaginalis* (7–21). Presently, the drug is available in the U.S. only in an oral dosage form (22). Parenteral dosage forms for a single injection are not available, presumably because of the relatively low solubility of metronidazole in water (~10 mg/ml at 25°). Sterile solutions for infusion are, however, available in other countries<sup>1</sup>. These preparations usually call for infusion of 100.0 ml of a 5 mg/ml solution. To overcome the solubility problem in formulating a parenteral solution, a prodrug approach was adopted in the present report. A water-soluble derivative was prepared and administered, which can be quantitatively converted to metronidazole by specific and/or nonspecific hydrolytic enzymes present in the body.

## BACKGROUND

For some drugs with alcoholic functional groups, hemiesters of dicarboxylic acids are derivatives with desirable aqueous solubility and facile enzymatic cleavage in the blood. Parenteral dosage forms of hydrocortisone<sup>2</sup>, methylprednisolone<sup>2</sup>, and chloramphenicol<sup>3</sup> are all hemiester

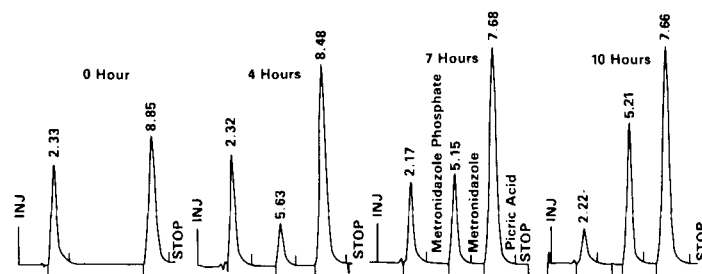
products. In the case of metronidazole, the monosuccinate ester is in the patent literature (23). One disadvantage associated with such monoesters of dicarboxylic acids is that they are not stable enough in aqueous solutions to provide a satisfactory shelflife for a parenteral solution (less than 10% decomposition within 2 years). Even without catalytic species from buffers, spontaneous hydrolysis of a simple ester prevents formulation in aqueous media. In the case of monosuccinates, hydrolysis is expected to be even faster because of the intramolecular catalysis from the remaining carboxylic acid and/or carboxylate anion (24).

The monophosphate of a drug with an alcoholic group carries two phosphoric acid functions with pKa values in the range of 2 and 6.5, and is freely soluble at physiological pH 7.4. In the past, phosphate esters have been used in preparing parenteral dosage forms of certain drug compounds (25) such as clindamycin, lincomycin, diethylstilbestrol, carboxybenzylpenicillin, hydroxysteroids, and trichloroethanol. Since these phosphate esters were found to be active *in vivo*, it was hoped that metronidazole phosphate also would be bioequivalent to or as bioavailable as the parent drug compound. The serum hydrolysis of metronidazole phosphate was studied to confirm that it regenerates metronidazole in the blood stream after intramuscular or intravenous injection within a reasonable period of time. Since phosphate esters are generally quite stable at neutral pH (26), metronidazole phosphate was expected to be stable enough to be prepared as a parenteral solution with an acceptable shelflife at an ambient temperature. The bioavailability of metronidazole from the phosphate ester prodrug was determined in rats.

## EXPERIMENTAL

**Solubility of Metronidazole and Metronidazole Phosphate**—A large excess of metronidazole was added to a series of 7-ml vials containing 5.0 ml of various solvents. They were shaken continuously for 24 hrs in a waterbath at 25 ± 0.2°, and filtered through disposable pipets with tips that were tightly packed with glass wool. An aliquot of the filtrate was evaporated under a nitrogen stream. The residue was redissolved in water and after proper dilution the concentration was determined from the absorbance<sup>4</sup> at 320 nm.

The apparent aqueous solubilities of metronidazole and its monophosphate also were determined at various pH values in a similar manner. In the latter case, the phosphate ester free acid was used; hence, at higher pH values, a downward pH drift was noted as the solubility equilibrium was attained. In such a case, the desired pH was maintained by intermittently titrating the sample with concentrated sodium hydroxide solution. In both cases, the concentration of solutes was determined by an



**Figure 1**—Reversed-phase HPLC analysis for the serum hydrolysis of metronidazole phosphate to metronidazole at 37°. The samples were obtained after 0, 4, 7, and 10 hr of hydrolysis at an initial concentration 0.50 mg/ml. Numbers by the peaks are the retention times in minutes. Picric acid served as an internal standard.

<sup>1</sup> Rhone-Poulenc (SPECIA), France.

<sup>2</sup> Solu-Cortef and Solu-Medrol, The Upjohn Co., Kalamazoo, Mich.

<sup>3</sup> Chloromycetin, Parke-Davis, Morris Plains, N.J.

<sup>4</sup> Beckman Model DB-G Spectrophotometer.

Table I—Solubility of Metronidazole at 25°

| Solvent            | Solubility, mg/ml         |
|--------------------|---------------------------|
| Water              | 9.50                      |
| Ethanol            | 5.00 <sup>a</sup>         |
| Methanol           | 32.20                     |
| Acetone            | 20.70                     |
| Benzene            | 0.65                      |
| Ethyl acetate      | 6.50                      |
| Acetonitrile       | 17.20                     |
| Ether              | 0.99                      |
| Chloroform         | 4.01                      |
| Methylene chloride | 4.12                      |
| Hexane             | $\sim 2.5 \times 10^{-3}$ |
| Dioxane            | 18.40                     |
| Tetrahydrofuran    | 17.70                     |

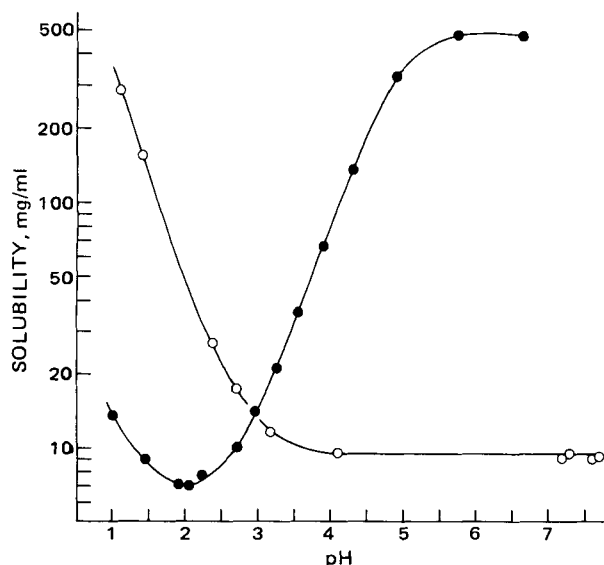
<sup>a</sup> "The Merck Index," 8th ed., Merck & Co., Rahway, N.J., 1968, p. 695.

HPLC procedure. Buffers used in this series of experiments were hydrochloric acid, chloroacetate, acetate, and phosphate systems.

**Synthesis of Metronidazole Phosphate (Method A)**—This method essentially followed the literature procedure in which 2-cyanoethyl phosphate was used in preparing phosphate esters (27). The barium salt of 2-cyanoethyl phosphates (16.16 g, 0.05 mole) was added to a suspension of a cationic exchanger<sup>5</sup> (70 ml in 150 ml water), and stirred until the solution was completed. The entire suspension was poured into a column containing the same resin (50 ml), and the column was eluted with water (300 ml). The eluent plus pyridine ( $\sim 30$  ml) was evaporated under vacuum at 40°, and the residue was dried further by evaporation with anhydrous pyridine. Finally the residue was dissolved in 50 ml of dry pyridine.

Metronidazole (3.42 g, 0.02 mole) and the 2-cyanoethyl phosphate stock solution (40 ml, 0.04 mole) were mixed and concentrated under vacuum. After being dried completely, dicyclohexylcarbodiimide (20.6 g, 0.1 mole)<sup>5</sup> in pyridine (180 ml) was added, and the reaction mixture was kept in the dark at room temperature for 2 days. Water (350 ml) was added and dicyclohexylurea was filtered off 2 hr later. The filtrate was taken up into a cationic exchanger suspension<sup>6</sup> (60 ml), stirred for 20 min, and filtered. After concentration, the filtrate was subject to a silica gel liquid chromatographic separation<sup>7</sup> using methanol–water–acetic acid (100:2:1) as the mobile phase. UV absorption of the eluent was continuously monitored at 320 nm. The fraction between 1.3 and 2.0 liter was combined and concentrated to 20 ml.

The solution was then titrated with  $\sim 31$  ml of 1.0 N KOH over 30 min



**Figure 2**—The pH dependence of the apparent solubility of metronidazole (O) and metronidazole phosphate (●) at 25°. The concentration of the latter was expressed in terms of its equivalence to metronidazole.

<sup>5</sup> Aldrich Chemical Co., Milwaukee, Wis.

<sup>6</sup> Dowex 50W, 20–50 mesh, H<sup>+</sup> form; Bio-Rad Laboratories, Richmond, Calif.

<sup>7</sup> Three Size C Silica Gel 60 prepacked columns from EM Laboratories.

Table II—pKa Values of Substituted Imidazoles<sup>a</sup>

| Compound                               | pKa               |
|----------------------------------------|-------------------|
| Imidazole                              | 6.953             |
| 1-C <sub>2</sub> H <sub>5</sub> -      | 7.300             |
| 1-CH <sub>3</sub> -5-NO <sub>2</sub> - | 2.130             |
| 1-CH <sub>3</sub> -4-NO <sub>2</sub> - | -0.530            |
| 2-CH <sub>3</sub> -                    | 7.851             |
| Metronidazole                          | 2.62 <sup>b</sup> |

<sup>a</sup> From ref. 31. <sup>b</sup> See text.

at 45° maintaining the pH at  $\sim 10$ –11. After another 45 min at 45°, the solution was neutralized with 1.0 N HCl. After evaporation, the solid residue was triturated with methanol and filtered. The filtrate was again evaporated to the crude product, which was recrystallized twice from 95% ethanol (3.23 g; 50% yield), mp  $>180^\circ$ ; NMR (methanol-*d*<sub>4</sub>):  $\delta$  7.85 (s, 1H, C-4), 4.5 and 3.9 (t, 2H each, —CH<sub>2</sub>—CH<sub>2</sub>— at N-1), and 2.5 (s, 3H, C-2) ppm; IR (Nujol)  $\nu_{\max}$ : 3200 (H<sub>2</sub>O), 1525 (—NO<sub>2</sub>), 1250 (phosphate), and 980 (phosphate) cm<sup>-1</sup>; Karl Fischer water 6.91%.

*Anal.*—Calc. for C<sub>6</sub>H<sub>8</sub>N<sub>3</sub>O<sub>6</sub>K<sub>2</sub>P·1.35 H<sub>2</sub>O: C, 20.50; H, 3.07; N, 11.95; O, 33.45; K, 22.24; P, 8.81. Found: C, 20.07; H, 3.14; N, 10.76; K, 23.04.

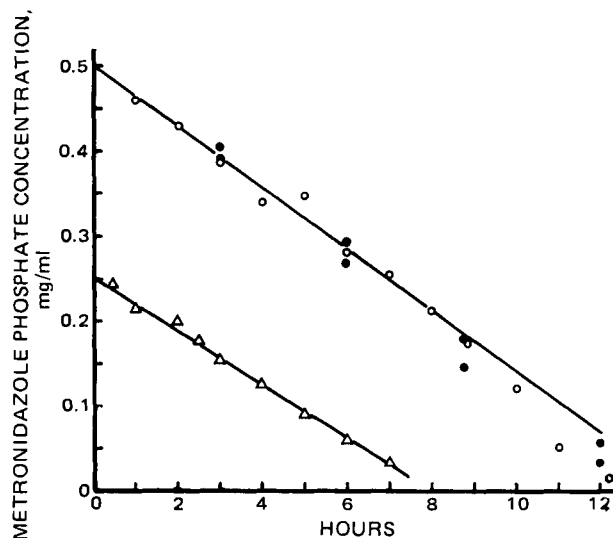
**Synthesis of Metronidazole Phosphate (Method B)**—Pyrophosphoryl tetrachloride was prepared following a literature procedure (28). Metronidazole (5 g, 0.029 mole) was dissolved in tetrahydrofuran (375 ml), stirred, and cooled to  $-25^\circ$ . The pyrophosphoryl tetrachloride (12 ml) was then added, and the resulting mixture was stirred at  $-25^\circ$  for 1 hr. The mixture was then poured into ice water (200 ml), stirred well, and the solvent was evaporated under vacuum. The resulting oil was triturated with acetonitrile, then seeded and stirred with 95% ethanol (200 ml) to crystallize 6.0 g of metronidazole monophosphate free acid (82% yield), mp 237–238°; IR (Nujol)  $\nu_{\max}$ : 3130, 2620, 2300, 1140, 1070, 945, and 865; equivalent weight 125 by titration.

*Anal.*—Calc. for C<sub>6</sub>H<sub>10</sub>N<sub>3</sub>O<sub>6</sub>P: C, 28.69; H, 4.01; N, 16.73; P, 12.34. Found: C, 28.70; H, 4.20; N, 17.04; P, 12.04.

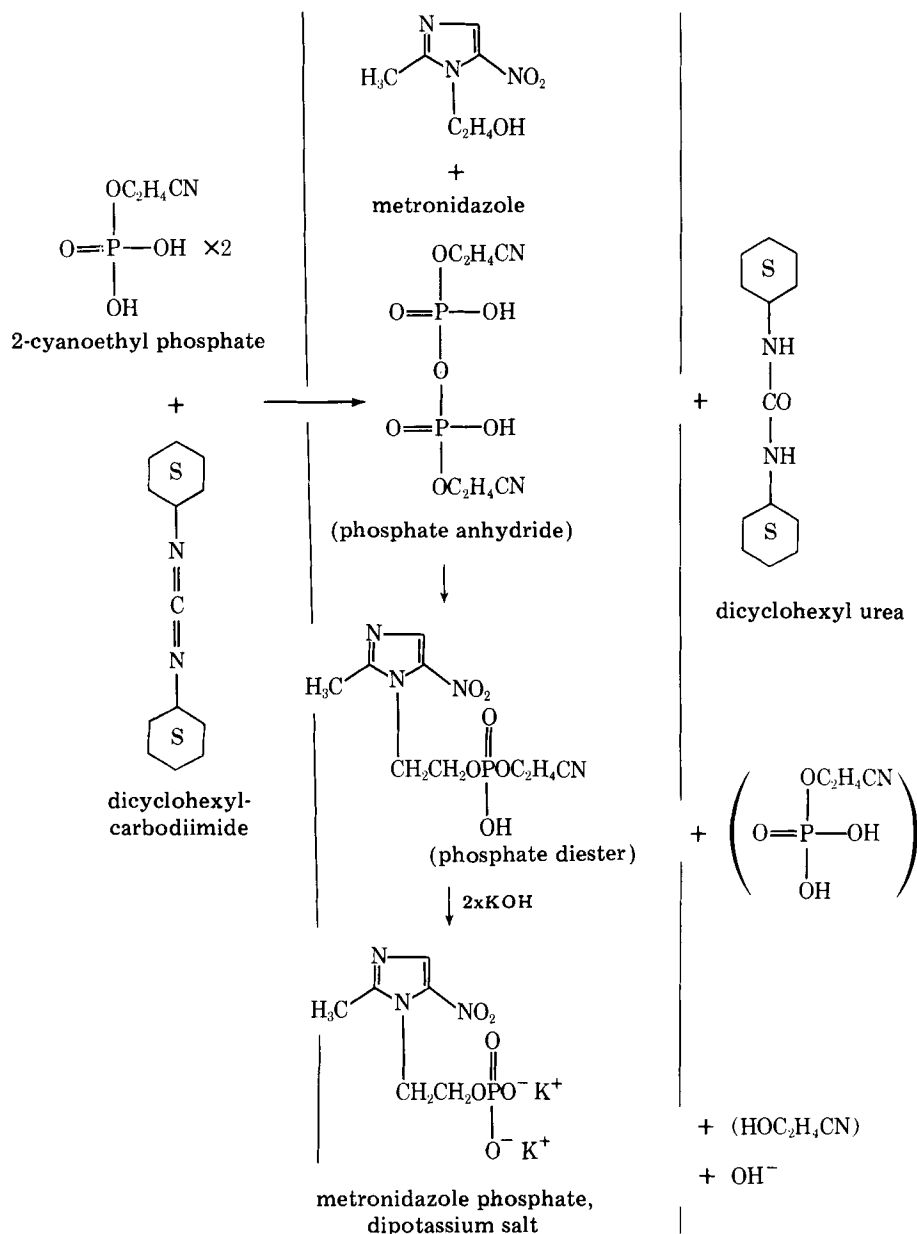
Metronidazole monophosphate free acid (4.35 g; 0.017 mole) was dissolved in water (40 ml) and titrated with 1.0 N KOH to pH 7.20. The resulting solution was freeze-dried to give 6.2 g white powder, which was recrystallized from 95% ethanol to give dipotassium salt hydrate.

*Anal.*—Calc. for C<sub>6</sub>H<sub>8</sub>N<sub>3</sub>O<sub>6</sub>K<sub>2</sub>P·4H<sub>2</sub>O: C, 17.98; H, 4.50; N, 10.48; P, 7.72. Found: C, 17.99; H, 4.06; N, 11.06; P, 7.88.

**Serum Hydrolysis of Metronidazole Phosphate**—To each of three 15-ml test tubes with a screw cap were added 0.50, 0.25, or 0.10 ml of a metronidazole phosphate stock solution in water (10.0 mg/ml) and 9.50, 9.75, or 9.90 ml human serum at 37°, respectively. The tubes were shaken immediately and kept at  $37 \pm 0.2^\circ$ . At proper intervals, 0.50-ml aliquots were transferred to a series of 10-ml centrifuge tubes containing 2.0 ml of methanol. After being shaken thoroughly, samples were kept at  $-20^\circ$  until analysis. Just prior to analysis, 0.05 or 0.20 ml of a picric acid stock solution in water (1.56 mg/ml) was added, mixed thoroughly, and centrifuged. The supernate was analyzed directly by HPLC. A typical res-



**Figure 3**—Serum hydrolysis of metronidazole phosphate at 37° at two initial concentrations. Data obtained from the human serum of two volunteers are represented as open and closed symbols, respectively.



Scheme I—Synthesis of metronidazole phosphate.

olution of metronidazole phosphate and metronidazole is shown in Fig. 1. The following chromatographic conditions were used with a commercially obtained column and equipment<sup>8</sup>: mobile phase, 0.10 M phosphate buffer (pH 7.0) and methanol (90:10); pressure, 1500 psi; flow rate, 0.50 ml/min; column temperature 50°;  $\lambda$ , 317 nm; attenuation, 0.08–0.16 absorbance unit; sample size, 5–9  $\mu$ l.

**Bioavailability Studies in Rats**—A single 40 mg/kg dose of metronidazole phosphate dipotassium salt was administered subcutaneously to each of three male rats weighing 190–210 g. These animals were fasted (except for water) for 24 hr prior to and during the experiments. Similarly, the parent compound, metronidazole, was administered to three rats. In both cases, the drugs were dissolved in 10% aqueous dimethylformamide.

At a given time after the drug administration, blood was withdrawn by clipping a portion of the tail. Microbiological assay disks<sup>9</sup> were saturated with the blood sample, placed on *C. perfringens*<sup>10</sup> seeded assay agar, and incubated anaerobically at 37°. The size of the inhibition zone was then measured to the nearest millimeter and the metronidazole con-

centration was determined from a standard curve. The area under a curve on the plot of blood concentration *versus* time was calculated by the trapezoidal rule using a digital computer.

## RESULTS AND DISCUSSION

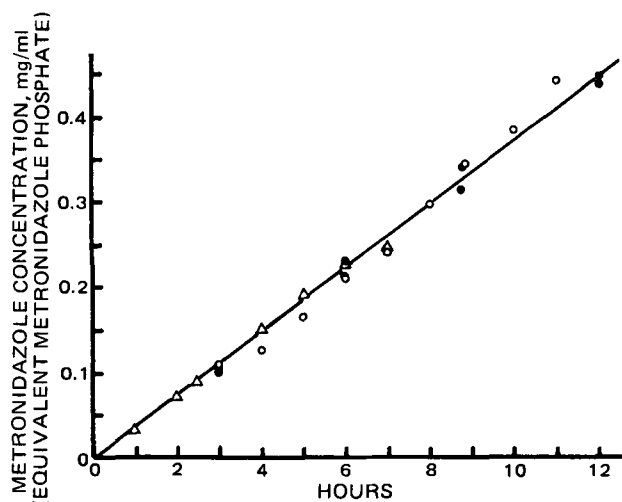
**Physical Properties of Metronidazole and Metronidazole Phosphate**—The solubility of metronidazole in various solvents is listed in Table I; the average of duplicate determinations is reported. In general, basic polar solvents such as tetrahydrofuran and dioxane dissolve metronidazole well, presumably through hydrogen binding. On the other hand, metronidazole phosphate was found to be freely soluble in water. Its dipotassium salt is also soluble in methanol and hot ethanol.

The pH dependence of the solubility of metronidazole at  $25 \pm 0.2^\circ$  is shown in Fig. 2, from which the apparent pKa of metronidazole was estimated as follows: from  $S = S_0 [1 + K_a/(H^+)]$ , in which  $S$  and  $S_0$  are the pH dependent apparent and intrinsic solubilities, respectively,  $S = 2S_0$  when  $(H^+) = K_a$  (30). A value of 2.62 was obtained for the pKa of the protonated N-1. Compared with imidazole (pKa  $\sim 7$ ), metronidazole is a much weaker base. This is because of the adjacent  $-\text{NO}_2$  group at C-5. Table II lists the pKa values of some structurally related compounds reported in the literature (31). As expected, electron-donating alkyl groups at N-1 or C-2 position of imidazole increase the pKa value but this effect is overridden by the adjacent  $-\text{NO}_2$  group (e.g., 1-methyl-5-ni-

<sup>8</sup> DuPont Model 830 HPLC unit was equipped with a DuPont Model 837 spectrophotometer, a Hewlett-Packard Model 3380A integrator, and an automatic injector valve described elsewhere (29). The column used was a Zorbax ODS column from DuPont (2.1 mm i.d.  $\times$  30 cm).

<sup>9</sup> S&S Disc (no. 740-E), Schleicher Schuell Co., Keene, N.H.

<sup>10</sup> *C. perfringens* was obtained from Center for Disease Control, Atlanta, Ga. In-house identification number UC-6054.



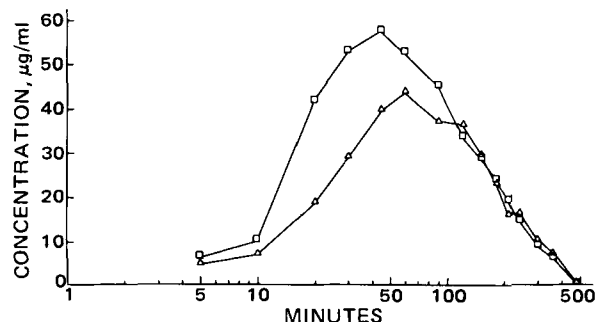
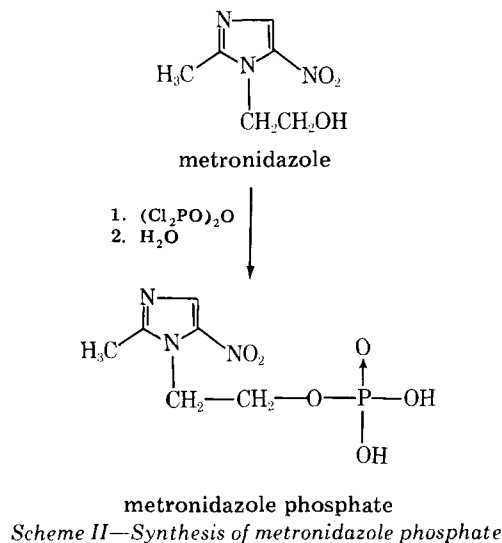
**Figure 4**—Appearance of metronidazole from the serum hydrolysis of metronidazole phosphate. Symbols are the same as in Fig. 3.

troimidazole). Interestingly, the electron-withdrawing effect through an induction effect appears less significantly than through a resonance interaction (e.g., 1-methyl-4- and 5-nitroimidazole).

Since the first pKa of the two remaining phosphoric acid functions should be close to 2 and since that of protonated N-1 is in the range of 2.5, metronidazole phosphate should predominantly exist as a zwitterion at pH ~2.5. Accordingly, the apparent solubility of the prodrug shows its minimum at pH 2 (Fig. 2). Note, however, that the solubility of the prodrug exceeds 730 mg/ml, equivalent to 500 mg of metronidazole/ml, at pH 7.0, approximately 50 times the metronidazole solubility. As pH increases further, the solubility of metronidazole phosphate increases again as the second phosphoric acid undergoes ionization. Thus, it was possible to formulate the prodrug at a concentration of metronidazole desired in clinical studies, in a desired volume, at a physiologically acceptable pH.

During the measurement of the melting point of metronidazole phosphate dipotassium salts, it was noted that the compound undergoes dehydration at ~90°. This process was seen clearly when a sample suspended in silicone oil was heated. Under microscope, the formation of a distinctive water phase from the dehydration was observed as the temperature approached 90°. Thermal gravimetric analysis, differential scanning calorimetry, and Karl Fischer titration were adopted for further characterization. After dehydration, the compound decomposed at ~180°. Such thermal behavior, dehydration of nonstoichiometric amount of water, and gradual decomposition, is commonly found among other salts of phosphate esters (32).

**Synthesis of Metronidazole Phosphate**—Method A described earlier employed that 2-cyanoethyl phosphate route in which dicyclohexylcarbodiimide was used as a dehydrative coupling reagent (Scheme I). The procedure is well established in the area of nucleotides (27) and was



**Figure 5**—Blood levels in rats following subcutaneous administration of metronidazole (□) or metronidazole phosphate (Δ).

adopted in the synthesis of lincomycin phosphate (32). The procedure was adopted initially in the synthesis of metronidazole phosphate primarily for a feasibility study. Method B, on the other hand, utilizes the high reactivity of pyrophosphoryl tetrachloride (28). This procedure (Scheme II) is simpler and more convenient than Method A.

**Biological Properties of Metronidazole Phosphate**—The hydrolysis rate of metronidazole phosphate to metronidazole, catalyzed by a number of enzymes, was determined by monitoring the appearance of the latter and the disappearance of the former in whole human serum at 37°. Throughout kinetic experiments, the mass balance was well observed.

As shown in Fig. 3, the disappearance of metronidazole phosphate in serum at 37° follows zero-order kinetics at two initial concentrations, 0.50 and 0.25 mg/ml, with rate constants 0.036 and 0.031 mg/ml/hr ( $1.10$  and  $0.95 \times 10^{-4} \text{ M hr}^{-1}$ ), respectively. For a sample with an initial concentration 0.10 mg/ml, no metronidazole phosphate was detected after 3 hr. Limited data obtained within the initial 3 hr appeared to follow first-order kinetics, but this remains to be confirmed.

Serum used for three runs of experiments and with an initial concentration of 0.50 mg/ml was obtained from the two healthy male volunteers. An identical rate constant appears to satisfy both series of data, indicating that the total enzyme activity towards metronidazole phosphate does not vary significantly between the two volunteers. Experiments with an initial concentration of 0.25 mg/ml produced a rate constant virtually identical to that obtained with an initial concentration of 0.50 mg/ml, leading to a conclusion that phosphatases and possibly other enzymes that cause the bioconversion are most likely saturated with the substrate at such high substrate concentration. Figure 4, where the appearance of metronidazole in three series of experiments is presented as a function of time, also produces a single zero-order rate constant, 0.037 mg/ml/hr ( $1.13 \times 10^{-4} \text{ M hr}^{-1}$ ) for the disappearance of the phosphate. This rate constant corresponds to 0.019 mg/ml/hr ( $1.13 \times 10^{-4} \text{ M hr}^{-1}$ ) in terms of the appearance of metronidazole. The ratio of molecular weight of metronidazole to that of the phosphate is 0.523. The average of three rate constants for the hydrolysis of the phosphate ester reported above, 0.035 mg/ml/hr ( $1.06 \times 10^{-4} \text{ M hr}^{-1}$ ), represents the maximum velocity in the apparent Michaelis-Menten kinetics (33).

As shown in Fig. 5, metronidazole phosphate produced blood levels of bioactivity in rats given 40 mg/kg subcutaneously (metronidazole equivalent) that were comparable to those produced by metronidazole when administered at the same dosage level. The maximum blood level produced by the phosphate ester was 43.3 μg/ml while that produced by metronidazole was 57.4 μg/ml, which occurred 60 and 45 min after administration, respectively. The corresponding ratio of total area under a curve was found to be 0.88:1.00. No conclusive explanation for the slightly (but significant at 95% confidence level) less area under a curve obtained from the phosphate ester than from metronidazole are given in the present report, although it is possible that the phosphate prodrug, being a highly ionic compound, may be readily eliminated intact through the kidney prior to or during the enzymatic conversion to the parent compound. In this aspect, it is interesting to note that the difference in bioavailability occurs during the initial 100 min and that the assumed elimination phase is nearly identical in both cases.

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# Dose-Dependent Pharmacokinetics of the Antihypertensive 2,3,4,4a-Tetrahydro-1H-pyrazino[1,2a]quinoxalin-5-(6H)-one in Dogs and Rats

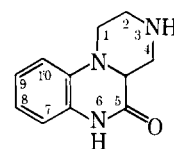
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**Abstract** □ A sensitive and reproducible GLC assay was developed for determining 2,3,4,4a-tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one (I) in biological fluids, utilizing the electron-capturing capability of the heptafluorobutyl derivative. After single 2.5- and 10-mg/kg oral and intravenous doses to three dogs, plasma concentration-time data for I were fitted to a biexponential equation and pharmacokinetic parameters were calculated. A dose-dependency for certain parameters, most notably total body clearance ( $Cl_T$ ), was indicated. The difference in  $Cl_T$  for the low and high dose was statistically significant. After single 5-, 25-, and 50-mg/kg intragastric doses were given to rats, the decline in plasma concentrations of I with time followed a monoexponential equation. As with dogs, there was a disproportionate change in kinetic parameters with increasing dose for rats. While simple Michaelis-Menten kinetics were not evident, nonlinearity in biotransformation (intrinsic clearance) appeared to be the cause for the dose-dependent pharmacokinetics.

**Keyphrases** □ 2,3,4,4a-Tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one—dose-dependent pharmacokinetics, dogs and rats □ Antihypertensive agents — 2,3,4,4a-tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one, dose-dependent pharmacokinetics, dogs and rats □ Pharmacokinetics—dose-dependent, 2,3,4,4a-tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one, dogs and rats

The compound 2,3,4,4a-tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one (I) is an antihypertensive agent that is effective in lowering blood pressure in hypertensive



dogs, cats, and rats<sup>1</sup>. Its synthesis has been described previously (1).

For metabolic disposition studies, single intravenous and oral doses of 2.5 and 10 mg/kg were given to normotensive male dogs and intragastric doses of 5, 25, and 50 mg/kg to normotensive male rats. From the resulting data, estimates of pharmacokinetic parameters were determined and the effect of dose on pharmacokinetics was investigated.

## EXPERIMENTAL

The hydrochloride salt and free base of I were used and 8-fluoro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2a]quinoxalin-5(6H)-one hydro-

<sup>1</sup> Dr. R. L. Wendt, Wyeth Laboratories Inc., Radnor, Pa., unpublished results.