Separation of Methotrexate and Nonmethotrexate Components in Rat Plasma

Keyphrases □ ^{3}H-Methotrexate—separation from labeled metabolites in rat plasma □ Plasma levels, ^{3}H-methotrexate—separation from labeled metabolites, rats

Sir:

Methotrexate (4-amino-N^{10}-methylpteroylglutamic acid) has been reported to be extensively metabolized by intestinal bacteria of mice and rats (1, 2) and to a lesser degree in man (3). Valerino (4) reported the separation of four different metabolites in the urine and feces of rats receiving tritiated methotrexate by gastric intubation and tentatively identified two as 2,4-diamino-6-carboxylpteridine and 2,4-diamino-6-methylpteridine. Although metabolites of methotrexate have been demonstrated in the urine and feces of animals, no reports of the separation of methotrexate from its metabolites in the plasma have appeared. A method for separation of methotrexate from its metabolites in plasma is essential for the necessary refinement of recently reported models of methotrexate pharmacokinetics (3, 5). These models have been derived from measurements of plasma concentrations of total radioactivity following administration of ^{3}H-methotrexate. This communication reports a simple method for the separation of ^{3}H-methotrexate from other labeled components in rat plasma.

Male Sprague–Dawley rats (450–500 g.) were injected with 25 mg./kg. of purified (6) ^{3}H-methotrexate *via* a cannula in the right external jugular. At specified times, 4 ml. of blood was drawn into a heparinized syringe and then centrifuged. Because of the volume of blood removed, no more than three samples were taken from any rat. Following centrifugation, the plasma was removed and the blood cells were washed with 2 ml. of normal saline and centrifuged. The saline supernate and plasma were combined and placed in a membrane filter cone[1]. The plasma water filtrate was obtained by centrifugation. The protein residue was washed with 2 ml. of water and again centrifuged. The combined filtrate was then frozen and lyophilized. The lyophilate, which contained ^{3}H-methotrexate, other labeled species, and plasma salts, was dissolved in a few drops of 95% ethanol and spotted on chromatography paper[2]. The chromatogram was developed using 0.1 *M* phosphate buffer (pH 7.0) as solvent. In control studies, methotrexate migrated as a single peak, R_f 0.50. The chromatographic strips were cut into sections, oxidized[3], and counted on a liquid scintillation spectrometer[4]. Following appropriate quench corrections, concentrations of ^{3}H-methotrexate and other labeled components in plasma were determined.

Table I—Percent of Total Label in Plasma Associated with Methotrexate and Nonmethotrexate Paper Chromatographic Peaks at Various Plasma Sampling Times[a]

Compound	Percent at 1 hr.	2 hr.	4 hr.	6 hr.
Methotrexate (R_f 0.5)	95	87	72	49
Nonmethotrexate (R_f 0.23)	2	5	25	50
Total	97	92	97	99

[a] Following a 25-mg./kg. i.v. dose.

Only one peak was found in the chromatogram of plasma samples taken from rats within the 1st hr. after injection. In samples taken from 1 to 6 hr. after injection of ^{3}H-methotrexate, two peaks were observed. The nonmethotrexate peak migrated with an R_f value of 0.23. Using this chromatographic system, Valerino (4) found all four metabolites in urine and feces to migrate with an R_f of 0.26–0.27. The proportion of label represented by this nonmethotrexate peak increased with time (Table I).

The total plasma levels of tritium label were corrected according to the percentage of total label under the

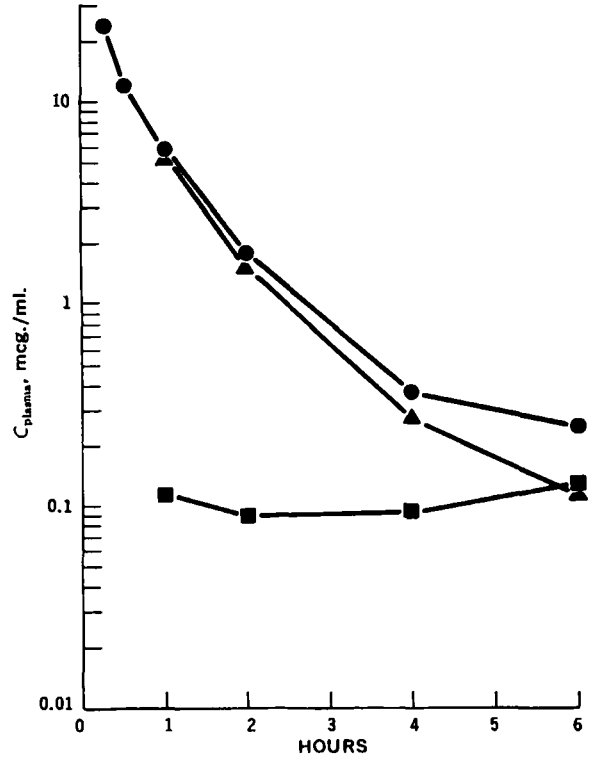

Figure 1—*Plasma concentration of ^{3}H-methotrexate equivalents with time. Key:* ●, *total;* ▲, *methotrexate; and* ■, *nonmethotrexate.*

[1] Amicon.
[2] Whatman No. 3MM.
[3] Packard sample oxidizer.
[4] Packard Tri-Carb.

methotrexate peak to give levels of actual methotrexate. As can be seen in Fig. 1, by 4 hr. the contribution of the nonmethotrexate component was very significant. Although the percentage of total label represented by the nonmethotrexate component increased with time, the actual concentration appeared to remain relatively constant.

Whether the nonmethotrexate component originates from metabolism of methotrexate by intestinal bacteria, by metabolism within the rat, from a trace contaminant in the purified ^{3}H-methotrexate solution for injection, or from a combination of these factors is unknown at this time.

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Received August 23, 1973.

Accepted for publication September 24, 1973.

Supported by Academic Senate research funds, University of California, San Francisco, Calif.

R. E. Kates acknowledges receipt of a U. S. Public Health Service Traineeship (5T01-GM00728-11).

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Inconsistencies in Rationale Underlying Official USP Dissolution Rate Specifications for Nitrofurantoin

Keyphrases ☐ Nitrofurantoin tablets and oral suspension—dissolution rate specifications with respect to compendial requirements ☐ Oral suspension, nitrofurantoin—dissolution rate specifications with respect to compendial requirements ☐ Dissolution rates, nitrofurantoin tablets and oral suspension—considerations with respect to compendial requirements

Sir:

Nitrofurantoin, 1-[(5-nitrofurfurylidene)amino] hydantoin, is an antibacterial agent used clinically to treat specific urinary tract infections. Physiocochemically, the drug is a weak acid (pKa 7.2) possessing relatively low aqueous solubility characteristics at pH values normally encountered in the various segments of the GI tract of man. As a result, it is not surprising that the drug displays a particle-size dependence in its dissolution rate (1) and rate and extent of absorption (bioavailability) in man (1–4).

USP XVIII (5) recognizes aqueous suspension and compressed tablet dosage forms of the drug, but only the monograph for nitrofurantoin tablets contains specifications for dissolution rate determinations. The requirement, contrary to that for other official drug tablet dosage forms, states that: "The time required for 60 percent of the labeled amount [50 or 100 mg.] of $C_8H_6N_4O_5$ in the Tablets to dissolve is not less than 1 hour, pH 7.2 phosphate buffer [900 ml.] being used as the *Dissolution Medium* and the basket being rotated at 100 r.p.m., and ...." Apparently, it is the intent of this unusual specification to ensure a *slow* rate of dissolution of nitrofurantoin from commercial tablet dosage forms in the GI fluids and thereby reduce both the maximum concentration of drug bathing the GI mucosa and present in the systemic circulation at any time. This, in turn, would presumably minimize the incidence of the major side effects of nitrofurantoin therapy in man, namely, locally (mucosal irritation) and/or systemically induced nausea and emesis (2, 6). Based on clinical observations of increased tolerance to capsules containing *macrosize* drug (80–200 mesh) as compared to tablets containing *microsize* (about 10 μ) drug (6), the rationale underlying the official dissolution rate specification for nitrofurantoin tablets appears, at first glance, to be sound, albeit totally arbitrary from a quantitative point of view. However, the requirement does not impose an upper dissolution rate limit and thus fails to ensure optimal bioavailability of nitrofurantoin from various brands of commercial tablets. Hence, the test does not reflect differences encountered in the *in vivo* absorption characteristics of nitrofurantoin from several different tablet formulations (7).

In addition, it appears quite inconsistent for the compendia, on the one hand, to be cognizant of the potential side effects of nitrofurantoin from tablet dosage forms but, on the other, not to provide for a dissolution rate specification for nitrofurantoin oral suspension USP. Apparently, it failed to recognize that the possible occurrence of adverse drug reactions from a suspension dosage form may be equal to, or even significantly greater than, that from a tablet dosage form. This conclusion can be readily appreciated from a consideration of basic biopharmaceutical principles (8)—*viz.*, that a relatively water-insoluble drug, administered orally in an aqueous suspension dosage form, generally dissolves, is absorbed, and, therefore, appears in the systemic circulation at a faster rate than can be normally achieved *via* administration of a compressed tablet dosage form.

While studying the rate of absorption and bioavailability of nitrofurantoin in man from different pharmaceutical dosage forms, the need arose to develop a *single* dissolution rate methodology applicable to both the official aqueous suspension and tablet dosage

Table I—*In Vitro* Dissolution Rates of Nitrofurantoin from Commercial Aqueous Suspension and Tablet Dosage Forms at 37°

Commercial Dosage Form	Mean Dissolution Half-Life, min.[a], at pH 7.20
Aqueous suspension	2.64 (±0.336)[b]
Compressed tablet	167 (±35.8)

[a] Determined from log-normal probability plots of dissolution rate data. Mean of five determinations. [b] Standard deviation in parenthesis.