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Fluorometric Determination of Cephradine in Plasma

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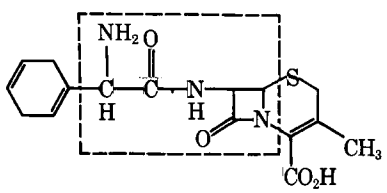
Abstract □ A fluorometric method was developed for the determination of cephradine in plasma. A fluorescent product is formed when samples of deproteinized plasma containing cephradine are heated for 3 hr at 100° and pH 1. The fluorescence is determined in sodium hydroxide solution (pH 13.5) at excitation and emission wavelengths of 350 and 445 nm, respectively. Only 0.1 ml of plasma is required, and concentrations of cephradine as small as 0.1 μg/ml may be determined. In plasma samples from a dog taken over a 10-hr period after an intramuscular injection of 250 mg of cephradine, essentially similar concentrations of cephradine were obtained by the fluorometric method and a standard microbiological bioassay.

Keyphrases □ Cephradine—fluorometric analysis, compared to microbiological bioassay, plasma □ Fluorometry—analysis, cephradine in plasma □ Antibiotics—cephradine, fluorometric analysis, plasma

Cephradine (I), 7-[D-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a semisynthetic cephalosporin (1). The concentration in biological fluids of cephradine, a poorly bound antibiotic, may be determined microbiologically (1). Colorimetric assays for cephalosporins (2) and cephradine (3) also have been reported.

During studies of the bioavailability of cephradine, it became desirable to develop a rapid, sensitive, chemical assay of the concentration of this antibiotic in plasma. Jusko (4) described a fluorometric assay for ampicillin that appeared to be specific for penicillins containing the structure enclosed by the dotted line in Structure I.

During initial attempts to adapt this assay to cephradine, plasma concentrations of cephradine less than 5–10 μg/ml could not be determined. Because the first step in the formation of a fluorescent product from ampicillin is the acid hydrolysis of the β-lactam ring, the greater stability of this ring in cephalosporins might be a major factor in the reduced sensitivity of the assay when applied to cephradine. The present sensitive analytical method for cephradine was developed by increasing the severity of the hydrolysis conditions.



I

EXPERIMENTAL

Reagents—The following reagents were used: 10% aqueous trichloroacetic acid solution, 4 *N* sodium hydroxide solution, and 0.2 *M* potassium chloride–hydrochloric acid buffer, pH 1.0. Glass-distilled water was used throughout, and the use of polyethylene containers was avoided (5).

Cephradine Standard Curve—Cephradine standards were added to control plasma. Because of possible variations in the water content of separate batches of cephradine, each cephradine standard was calibrated microbiologically. All weights were expressed as micrograms or milligrams of microbiological activity. Aliquots of a stock solution of cephradine in water (100 μg/ml) were diluted with plasma to prepare a suitable range of working standards as well as a blank. Three 0.1-ml aliquots of each standard were carried through the procedure with each batch of plasma samples.

Microbiological Method—The concentration of cephradine in plasma samples was determined microbiologically by an agar-diffusion method, with *Sarcina lutea* (ATCC 9341) as the test organism.

Animal Experiment—Cephradine for injection (250 mg) was injected into the semitendinosus muscle of a male beagle dog (10.6 kg). Plasma was obtained at intervals for 10 hr, and the cephradine content of each sample was determined by both the spectrofluorometric and microbiological methods.

Fluorometric Assay Procedure—Fluorescence was measured[1] at excitation and emission wavelengths of 350 and 445 nm, respectively. The instrument was zeroed with a reagent blank and set to read 90% of full-scale deflection against a suitable cephradine standard, depending on the range of concentrations expected in the unknowns.

[1] Farrand Mark I spectrofluorometer.

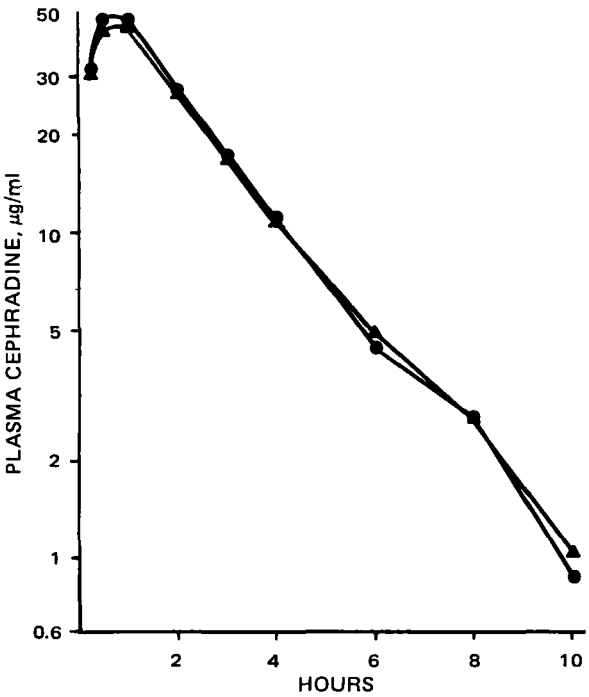


Figure 1—*Concentrations of cephradine in plasma from a male beagle dog after the intramuscular injection of 250 mg of cephradine. Key: ●, fluorometric assay; and ▲, microbiological assay.*

Duplicate samples were prepared by transferring 0.1-ml aliquots of each plasma sample to 2-ml centrifuge tubes. Trichloroacetic acid solution (1.0 ml) was added to each tube, the contents were mixed well, and the tubes were centrifuged for 5 min at 800×*g*. Portions (0.1 ml) of the supernate were transferred to graduated Pyrex centrifuge tubes, followed by 1.0 ml of pH 1.0 potassium chloride–hydrochloric acid buffer.

After mixing, each tube was capped with a glass marble and incubated at 100° for 3 hr. When the tubes had cooled to room temperature, the volumes were made up to 2 ml with distilled water. Sodium hydroxide solution (0.5 ml) was added to each tube, and the contents were mixed well. The fluorescence of each standard and unknown was determined, a standard curve was prepared, and the concentration of cephradine in each sample was read directly from the standard curve.

RESULTS AND DISCUSSION

The fluorescence of cephradine was linear over the range of 0.1–100 μg/ml of plasma. A sample of control plasma spiked with 0.1 μg of cephradine/ml gave a reading of 0.135 fluorescence unit, as compared to a reading of 0.01 unit for the control plasma. The coefficients of variation for triplicate samples of cephradine at different concentrations in plasma were 13% at 0.1 μg/ml, 5.8% at 0.5 μg/ml, 3.7% at 1.0 μg/ml, and 2.7% at 2.0 μg/ml. The coefficient of variation of the microbiological assay for five replicate samples was 3.5% at 0.5 μg/ml[2]. Although it appeared to be more precise, the microbiological assay required a much larger volume of plasma and was more time consuming than the fluorometric method.

Because they contain the structure enclosed by the dotted line in Structure I, cephalexin, ampicillin, and epicillin also react in this procedure, but other common cephalosporins and penicillins do not. Fluorescent material might possibly be derived from the hydrolysis products of cephradine. However, this is not a problem in *in vivo* studies, because cephradine is excreted unchanged (1). Both spectrofluorometric and microbiological methods gave similar results for the concentration of cephradine in plasma after its intramuscular injection (Fig. 1).

Conversion of cephradine to the fluorophore was not complete after the 3-hr incubation. Since most of the observed cephradine concentrations were in excess of 1 μg/ml of plasma, the operating convenience of the 3-hr hydrolysis time outweighed any gain in sensitivity that might have been obtained by prolonging the incubation. The hydrolysis time may be increased at the discretion of the investigator. Care should be taken that all standard and unknown samples are subjected to identical conditions of hydrolysis and cooling.

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