The physical parameters for these conditions are summarized in Table II. Figure 2 shows the amount of water lyophilized *versus* square root of time curves generated by the steady-state expression described in Eq. 36, whereby the surface diffusion consideration is neglected. As can be seen, the lyophilization rates are faster at −20° than at −30°. Lyophilization is completed in 7.7 hr at the surface temperature of −20° and in 13 hr at −30°. The linear profiles of the receding boundary with the square root of time according to Eq. 35 are observed in Fig. 3.

Future studies will be directed toward the experimental verification of the interdependence of the variables described in this paper.

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NOTES

Flame-Ionization GLC Assay for Fluorouracil in Plasma of Cancer Patients

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Received December 21, 1978, from the *Laboratoria voor Medische Biochemie en voor Klinische Analyse, Faculteit van de Farmaceutische Wetenschappen, Rijksuniversiteit, Academisch Ziekenhuis, 135 De Pintelaan, 9000-Gent, Belgium.* Accepted for publication February 20, 1979.

Abstract □ A rapid and specific flame-ionization GLC method was developed for the determination of plasma fluorouracil. The chloro analog is used as the internal standard. The method involves the isolation of both the drug and the internal standard from plasma on a strong anion-exchange column at pH 10. Elution is performed with acetic acid in methanol. The evaporated eluate is dissolved in tetrahexylammonium hydroxide. An aliquot of the resulting solution is introduced directly into the gas chromatograph, where conversion to the bishexyl derivatives and subsequent separation take place. The extraction recovery from blank plasma, to which fluorouracil was added, was 96.8 ± 2.4% (*SD*). Linearity was proven in the range from 0 to 25 μg/ml, whereas the detection limit of the method was estimated at about 2 μg/ml of plasma. The within-run precision was determined at three different fluorouracil levels. To demonstrate method applicability, plasma samples obtained from cancer patients to whom 1 g of fluorouracil had been administered intravenously were analyzed.

Keyphrases □ GLC, flame ionization—analysis, fluorouracil, human plasma, *in vivo* □ Antineoplastic agents—fluorouracil, flame-ionization GLC analysis, human plasma, *in vivo* □ Fluorouracil—analysis, flame-ionization GLC, human plasma, *in vivo*

Fluorouracil has been used for several years in the chemotherapeutic treatment of breast, stomach, and colon carcinoma. Different investigators administer fluorouracil in accordance with their own experience, using various dosage schedules (1–4). Systematic observation of fluorouracil disposition as a function of dosage schedule and route of administration in several patients would be valuable. Therefore, a rapid, sensitive, analytical method for fluorouracil determination in numerous biological samples is needed.

Besides the microbiological bioassays (5, 6), few chemical methods have been published. The spectrophotometric assay for fluorouracil (5) lacks specificity and sensitivity. GLC methods were reported using flame-ionization detection (7, 8), electron-capture detection (9), and multiple-ion detection (10–12). Most of these methods are based

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Table I—Fluorouracil Assay Precision

Plasma Level, μg/ml	$\bar{x}$, μg/ml	CV, %	n
10	10.16	4.79	24
4	4.27	9.97	16
2	2.72	10.53	20

on the chromatography of silyl drug derivatives (7–10) and on the use of internal standards such as anthracene (8) and thymine (9, 11). These standards are structurally unrelated to the compound to be assayed, and thymine is a possible endogenous compound. The mass fragmentographic method allows determination of plasma fluorouracil levels down to 2 ng/ml (12) but requires expensive apparatus.

The present work describes a simple and specific method for the determination of plasma fluorouracil levels. The procedure can be performed on a routine basis for clinical studies of patients receiving fluorouracil therapy.

EXPERIMENTAL

Reagents—A pH 10 carbonate buffer (ionic strength 0.1) was prepared by mixing 25.0 ml of 1 *M* $NaHCO_3$ with 50.0 ml of 0.5 *M* Na_2CO_3 and diluting to 1000 ml with double-distilled water.

A 0.2 *M* tetrahexylammonium hydroxide solution in methanol was prepared as described previously (13).

All other chemicals and solvents were analytical reagent grade.

Extraction from Plasma—Blood samples were collected in heparinized tubes, mixed, and centrifuged to allow plasma separation. The plasma was frozen until assayed. An aliquot of 1.0 ml of plasma, 100 μl of internal standard solution (20.0 mg of chlorouracil[1]/100 ml of methanol), and 4.0 ml of pH 10 carbonate buffer were mixed in a 15-ml centrifuge tube. The sample was transferred to a glass column packed with anion-exchange resin[2] (2 cm × 0.6 cm i.d.).

Prior to use, the column was washed with 10 ml of 0.3 *M* acetic acid in methanol, and the resin was equilibrated with 10 ml of pH 10 carbonate buffer. The entire sample was put through the resin bed. The column was consecutively washed with 10 ml of water and 10 ml of methanol. Both the drug and the internal standard were eluted with 10 ml of 0.3 *M* acetic acid in methanol. The eluate was evaporated to dryness in a water bath at 50° under a nitrogen stream.

Derivatization—The residue obtained after evaporation was dissolved into 25 μl of 0.2 *M* tetrahexylammonium hydroxide in methanol. An aliquot was introduced in the gas chromatograph[3] injection port.

GLC and Quantitation—The hexyl derivatives were chromatographed on a silanized glass column (1.8 m × 0.2 cm i.d.) packed with 3% Dexsil-300[4] on 80–100-mesh Gas Chrom Q. A flame-ionization detector was used. Nitrogen was the carrier gas at a linear velocity of 7 cm/sec.

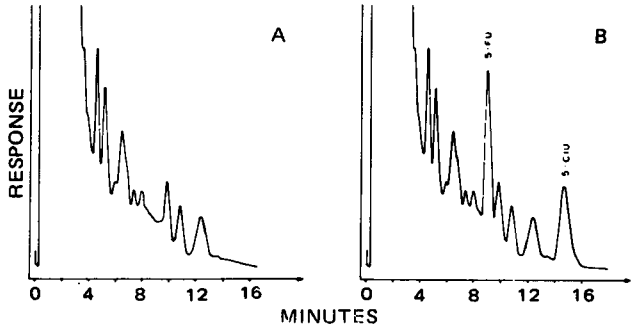

Figure 1—*GLC of 1 ml of drug-free plasma (A) and of 1 ml of plasma spiked with 10 μg of fluorouracil (B).*

[1] Calbiochem A. G., Lucerne, Switzerland.
[2] AG 1-X4 (Cl^-), 100–200 mesh, Bio-Rad, Richmond, Va.
[3] Hewlett-Packard model 5750G.
[4] Supelco Inc., Bellefonte, Pa.

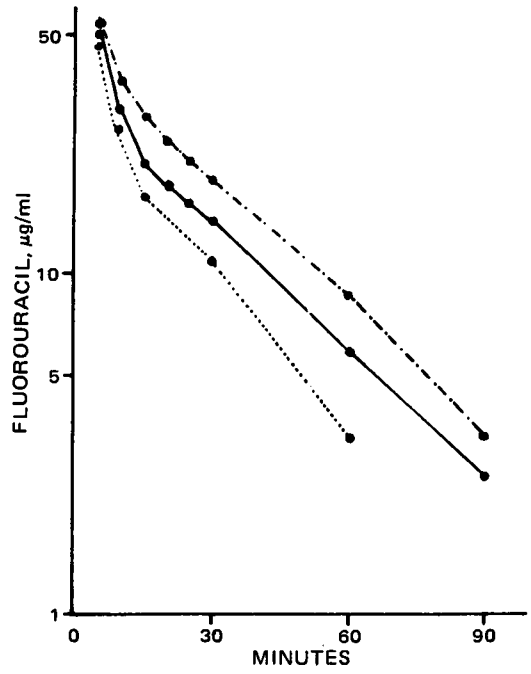

Figure 2—*Plasma fluorouracil concentration in Patient B (—), Patient VH (. . .) and Patient VW (---) after administration of a single 1-g intravenous dose.*

Hydrogen and air flows were adjusted to optimum sensitivity. The injection port, oven, and detector temperatures were 270, 230, and 250°, respectively.

Quantitation was accomplished by using calibration curves obtained from standard fluorouracil[5] solutions in methanol or from blank plasma to which known amounts of fluorouracil[5] had been added.

RESULTS AND DISCUSSION

The problems concerning the isolation of fluorouracil, floxuridine, and fluorouridine from aqueous solutions and the anion-exchange extraction procedure were discussed in previous papers (12, 14, 15).

Flash alkylation, based on the Hofmann degradation (16), converted both fluorouracil and chlorouracil into hexyl derivatives suitable for GLC analysis. The derivatives were identified by combined GLC–mass spectrometric[6] analysis as *N,N'*-dihexyl derivatives; the spectral characteristics for the fluorouracil derivative were: *m/e* (relative intensity) 298 (7.1) M^+, 215 (54.1) $C_{10}H_{16}N_2O_2F^+$ or $M - C_6H_{13} + 2H$, and 131 (65.5), $C_4H_4N_2O_2F^+$ or $M - 2C_6H_{13} + 3H$.

Figure 1 shows the gas chromatograms obtained from 1 ml of drug-free plasma (A) and from 1 ml of the same plasma to which 10 μg of fluorouracil (5-FU) and 10 μg of chlorouracil (5-ClU) had been added (B). Because of interference from endogenous compounds, the detection limit in plasma was ~2 μg/ml.

Blank plasma samples to which 0–25 μg of fluorouracil/ml had been added were taken through the entire procedure. A linear relationship existed between the peak height ratios of fluorouracil to chlorouracil and the plasma fluorouracil concentration (regression line: $y = 0.113x + 0.036$, $r = 0.9996$).

The extraction recovery was determined by analyzing plasma samples spiked with a known amount of fluorouracil and adding the internal standard to the column eluate. The fluorouracil concentration was calculated from a calibration curve obtained from standard fluorouracil and chlorouracil solutions in methanol and submitted to flash hexylation under the circumstances outlined. An extraction recovery of 96.8 ± 2.4% (*SD*) was calculated.

Three plasma pools containing various amounts of fluorouracil were analyzed on the same day (Table I). The values for the coefficients of variation (*CV*) indicate that the overall method reproducibility is satisfactory.

[5] Sigma Chemical Co., St. Louis, Mo.
[6] LKB model 9000S; 3% Dexsil-300 (1.8 m × 0.2 cm i.d.) Gas Chrom Q (100–120 mesh); helium flow, 30 ml/min; 20 ev; injector, oven, and separator temperatures of 260, 230, and 275°, respectively.

Method accuracy was checked by analyzing some samples with the GLC method and with a previously described UV method (17). The results were plotted against each other and correlation analysis revealed a correlation coefficient, r, of 0.9960 (regression line: $y = 1.028x + 0.203$).

Plasma samples collected at different time intervals from six cancer patients to whom 1 g of fluorouracil had been administered intravenously were analyzed. Control runs with plasma blanks, collected before the drug administration, showed no major interfering peaks eluting in the regions corresponding to the drug or to the internal standard.

Figure 2 shows data obtained from three representative patients. In all cases, the fluorouracil concentration leveled off rapidly, with no measurable plasma levels occurring after 60 min and for three patients after 90 min. After an extremely short distribution phase, the drug appears to be eliminated by a logarithmic linear phase. The applicability of the method to these patients indicates that the procedure is suitable for clinical pharmacological studies on fluorouracil.

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Influence of Cetylpyridinium Chloride on Corneal Permeability to Penicillin

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Received January 12, 1979, from the **Department of Ophthalmology and the* ‡*Department of Physiology, Medical College of Georgia, Augusta, GA 30901.* Accepted for publication March 14, 1979.

Abstract □ The epithelial surface or the deepithelialized anterior stromal surface of isolated rabbit corneas was perfused for 3 hr with ^{14}C-penicillin in 25 m*M* Ringer–bicarbonate solution with or without 1% albumin and with or without 0.02% cetylpyridinium chloride. The intact epithelium acted as a barrier to penicillin and impeded the flux rate by 66% when compared to the flux rate across the deepithelialized cornea. The presence of 0.02% cetylpyridinium chloride increased the penicillin flux rate across corneas with an intact epithelial layer to that of deepithelialized corneas. Cetylpyridinium chloride, 0.02%, had no effect on penicillin flux across deepithelialized corneas. The penicillin flux rate across corneas, with or without epithelium, was increased slightly following the inclusion of 1.0% albumin in the bathing solution. The flux rates across deepithelialized corneas in the presence of albumin, with or without cetylpyridinium chloride, were similar to fluxes found in the absence of albumin. Albumin–penicillin "binding" was not a significant factor in impeding penicillin flux, and this binding apparently was not altered by cetylpyridinium chloride. The surfactant appeared to alter epithelial permeability physiologically.

Keyphrases □ Cetylpyridinium chloride—effect on corneal permeability to penicillin, effect on epithelium □ Surfactants—cetylpyridinium chloride, effect on corneal permeability to penicillin, effect on epithelium □ Benzylpenicillin—corneal permeability, effect of cetylpyridinium chloride

The penetration efficiency of most topically applied drugs is very small, but penicillin penetration is especially poor (1, 2). Poor intraocular drug levels have been attributed (3) both to rapid drug removal by tears and to drug binding to the tear protein, thus rendering the drug biologically unavailable. Inclusion of 0.02% cetylpyridinium chloride with pilocarpine nitrate when applied topically to the rabbit eye caused 10 times the miotic effect of the same amount of pilocarpine without cetylpyridinium chloride. Previous investigators (4) hypothesized that cetylpyridinium chloride was a competitive inhibitor of pilocarpine protein binding and that the cetylpyridinium chloride allowed a higher percentage of drug to be unbound and bioavailable.

Cetylpyridinium chloride, when applied to the cornea, increased fluorescein penetration across the cornea; transmission electron microscopy revealed increased intercellular spaces in the superficial epithelial layer and lysis of the outermost cell membranes (5). Scanning electron microscopy revealed a loss of epithelial microvilli and microplicae, a process accompanied by surface pitting, which exposed deeper epithelial cells (6).

The purpose of this investigation was to delineate further the mechanism of cetylpyridinium chloride in increasing drug penetration into the eye by examining its effects on the corneal flux rate of a known albumin-bound drug.

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