may be adulterated or diluted during manufacture. When the ginseng powder is reduced to 40 or 60 mesh in the granulation, microscopic evaluation becomes less confirmatory. However, panaxoside levels should still be detected. The absence of detectable amounts of panaxosides requires further investigation into the manufacturing of ginseng tablets.

Granules and powders for the preparation of ginseng water infusions yielded panaxoside levels in the low region (0.1–0.4% of product weight).

Extracts yielded surprisingly low panaxoside levels, probably because of the relatively short shelflife of the opened extracts. The cosmetic tested yielded 0.3% panaxoside, within the recommended level of 0.25–0.5% for creams and rinses (15).

The TLC pattern of the roots can be correlated with the patterns of the powder-filled capsules and extracts and, to a lesser extent, the slurry-filled soft gelatin capsules. Most roots present at least four detectable panaxosides. Six-year-old roots show more extensive patterns and may yield up to nine panaxosides. All root samples tested yielded Rb1, while four of five yielded Rc, Re, and Rf. A similar pattern was seen with the capsule and extract products.

It is difficult to explain the tremendous variation among ginseng products. The wide range of total panaxoside content may be a function of the amount of ginseng root incorporated into the product. The lack of a consistent pattern of individual panaxosides in these products requires further clarification. The possibility of panaxoside loss during the manufacture of ginseng tablets is under investigation.

In view of the high prices of these products, there is a need for regulations regarding the control of commercial ginseng products.

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Use of a Nitrogen Detector for GLC Determination of Fluorouracil in Plasma during Single- and Combined-Agent Chemotherapy

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Abstract □ A GLC assay for fluorouracil was developed and used to monitor plasma drug levels in patients on both single- and combined-agent chemotherapy. Fluorouracil is extracted from plasma, derivatized by flash methylation, and estimated using a thermionic nitrogen–phosphorus detector. The GLC determination was accurate at concentrations as low as 0.1 μg/ml of human plasma. Other drugs commonly used in combination with fluorouracil did not interfere with the assay.

Keyphrases □ Fluorouracil—nitrogen-detector GLC analysis in plasma □ GLC, nitrogen detector—analysis, fluorouracil in plasma □ Antineoplastic agents—fluorouracil, nitrogen-detector GLC analysis in plasma

Since its synthesis in 1957, fluorouracil has been used extensively as an antimetabolite in the treatment of cancer, particularly of the breast and GI tract (1). More recently, it has been widely used in multiple-agent chemotherapy (2, 3). Despite this continued usage, the clinical response is often inconsistent, and a variety of empirical dosage schedules are still employed. Therefore, to optimize drug schedules for individual patients, it would be useful to relate the time course of fluorouracil in plasma, both alone and in combination with other cytotoxic drugs, to clinical response.

Most methods for measuring fluorouracil employ GLC techniques (4–6) or assay by analysis of microbial kinetics (7). The present study describes a GLC method with nitrogen detection capable of measuring fluorouracil in combination with other cytotoxic agents without interference.

EXPERIMENTAL

Materials—Fluorouracil[1] was used both in the solid form and as a solution for injection. Thymine[2], the internal standard, was obtained as the anhydrous solid. Trimethylanilinium hydroxide[3], 0.2 *M* in methanol, was the derivatizing agent. Buffer salts[4] and solvents[4] were analytical reagent grade.

GLC Conditions—A gas chromatograph[5] equipped with a thermionic

[1] Roche Products Ltd., Welwyn Garden City, Herts., England.
[2] Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey, England.
[3] Pierce and Warriner (UK) Ltd., Chester, Cheshire, England.
[4] B.D.H. Chemicals Ltd., Poole, Dorset, England.
[5] Model F30, Series III, Perkin-Elmer Ltd., Beaconsfield, Bucks, England.

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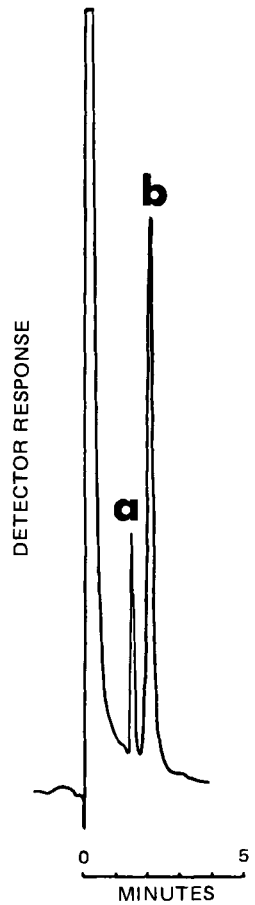

Figure 1—*Gas chromatogram showing fluorouracil (peak b, retention time = 2.00 min) and the internal standard, thymine (peak a, retention time = 1.45 min).*

nitrogen–phosphorus detector was used. A 2-m glass column packed with 0.75% Carbowax 20M–5% potassium hydroxide on 80–100-mesh Chromosorb G[6] was conditioned by temperature programming at 1°/min from 100 to 230° and maintained at 230° for 48 hr under low helium flow. The operating temperatures were: column, 210°; and injector and detector, 300°. Helium, the carrier gas, was maintained at a flow rate of 30 ml/min. The flow rates of air and hydrogen were optimized at 100 and 6 ml/min, respectively. Under these conditions, the retention times were 2.00 min for fluorouracil and 1.45 min for thymine (Fig. 1).

Procedure—Samples of 0.1–1 ml of plasma containing fluorouracil were diluted to 1 ml, where necessary, with blank plasma. This 1 ml of plasma was further diluted to 4 ml with 0.33 *M* phosphate buffer, pH 6.8, to give a final buffer concentration of 0.25 *M*. The mixture was heated in a water bath at 100° for 5 min, and the protein precipitate was removed by centrifugation at 3000×*g* for 5 min. A 2-ml aliquot of the supernate was removed and extracted by mixing with 25 ml of 16% propanol in ether for 1 min.

A 20-ml volume of the upper layer was removed and evaporated under nitrogen at 60° in a water bath. The dry residue was redissolved in 2 ml of methanol containing 0.5 or 1.0 μg of thymine as the internal standard. Then the mixture was transferred to a smaller tube and evaporated as previously described. The resultant residue was dissolved in 100 μl of 0.01 *M* trimethylanilinium hydroxide, and 1 μl was injected onto the column.

Standard curves over the ranges of 0.1–5.0 and 2–20 μg of fluorouracil/ml, containing 0.5 and 1.0 μg of thymine/ml, respectively, were prepared by extracting blank plasma and were linear.

Plasma samples not assayed immediately were stored at −20° for up to 4 weeks without any decrease in the measured fluorouracil levels.

RESULTS AND DISCUSSION

The described procedure can measure plasma fluorouracil levels accurately down to 0.1 μg/ml and is suitable for monitoring the time course of drug disappearance after oral and intravenous therapy. This method offers several advantages over previously published methods. It does not use contaminating silyl reagents for derivatization, thus obviating the need for frequent detector cleaning. It does not require the complex instrumentation of mass spectrometry, because use of the nitrogen detector increases the sensitivity of the assay 50–100 times in comparison with the responsiveness of the flame-ionization detector. Sensitivity was increased still further by adapting the extraction procedure of Hillcoat *et al.* (6) to omit the ether wash stage. Recovery of fluorouracil was 88 ± 5% (mean ± *SD*, n = 12), measured over the range of 0.2–10 μg of fluorouracil/ml of plasma. The coefficient of variation of the analyses was ±5.6%.

Unlike the mass spectrometric method, use of a nitrogen detector requires complete separation of the fluorouracil from the internal standard, thymine. Various stationary phases, over a wide range of polarity, were investigated to achieve this separation. The most suitable, 0.75% Carbowax 20M–5% potassium hydroxide, separated the two compounds with minimal retention times (Fig. 1).

The specificity of the assay in the presence of other cytotoxic drugs commonly used with fluorouracil was assessed in patients receiving 5-day cycles of quadruple chemotherapy. The cytotoxic regimen consisted of fluorouracil, cyclophosphamide, methotrexate, and vincristine, given in varying combinations intravenously over 3-min periods on different days[7]. Plasma samples were collected immediately after drug administration and were analyzed for fluorouracil. No interfering peaks attributable to cytotoxic agents other than fluorouracil were seen on the chromatograms.

The method is rapid, the compounds being eluted from the column in less than 3 min. It also does not require complex instrumentation, and it is specific for fluorouracil even in the presence of other antineoplastic drugs.

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