Equations for Estimation of First-Pass Effect and Apparent Distribution Volume of Drug with Incomplete Oral Absorption and Partial Renal Excretion

Keyphrases □ First-pass effect—estimation and distribution volume, drugs with incomplete oral absorption and partial renal excretion □ Distribution volume—estimation and first-pass effect, drugs with incomplete oral absorption and partial renal excretion □ Absorption, GI, incomplete—estimation of first-pass effect and distribution volume □ Excretion, renal, partial—estimation of first-pass effect and distribution volume

To the Editor:

Equations assuming complete absorption have been used to estimate the degree of pulmonary (1) and hepatic (2, 3) first-pass effect and the apparent distribution volume (1, 3) after oral dosing of drugs having partial pulmonary and renal excretion. The purpose of this communication is to present general equations that can be used when a drug is known to be absorbed only partially from the dosage form.

Based on the previously derived equations (1), the extent of the first-pass effect or the hepatic extraction ratio, designated as f_m, is:

$$f_m = \frac{(F_m)(F)(\text{dose})}{(F)(\text{dose}) + (HFR)(AUC_\infty)} \quad \text{(Eq. 1)}$$

where F_m is the hepatically metabolized drug fraction after intravenous administration or after entry into the general circulation through other routes of administration, F is the dose fraction available for GI absorption from the dosage form, HFR is the hepatic blood flow rate, and AUC_∞ is the total area under the blood concentration *versus* time curve at infinite time.

The apparent volume of distribution, V_d, based on the area method can be estimated by:

$$V_d = \frac{(F)(1 - f_m)(\text{dose})}{(K_t)(AUC_\infty)} \quad \text{(Eq. 2)}$$

where K_t is the first-order rate constant of the terminal postabsorption and postdistribution phase.

Similar equations can be derived for drugs that are partially absorbed and partially excreted from the lung and kidney.

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N-Benzoyl Derivatives of Amino Acids and Amino Acid Analogs as Inhibitors in Microbial Antitumor Screen

Keyphrases □ Antineoplastic agents, potential—amino acids, *N*-benzoyl derivatives, microbial screen □ Amino acids, *N*-benzoyl derivatives—potential antineoplastic agents, microbial screen

To the Editor:

For some time, we have been studying the *N*-acylated derivatives of amino acids and amino acid analogs as possible antitumor agents (1–3). Certain chloroacetylated (1, 3) and trifluoroacetylated (4) amino acids and amino acid analogs were found to be active inhibitors of the growth of a microbial system selected for antitumor screening. The degree of inhibition ranged from about 20 to 50%.

With the hope of increasing the activity of these compounds by giving them more lipophilic character, the benzoyl and ring-substituted benzoyl derivatives were prepared. The acylation was accomplished by the conventional Schotten–Baumann procedure (5). The purity of these compounds was ascertained by elemental analysis, melting-point determination, optical rotation determination where applicable, and Van Slyke nitrous acid determination of the primary amino nitrogen (6). The microbiological assay system utilized was *Lactobacillus casei* (7469) in a riboflavin-supplemented riboflavin assay system (3).

Mercaptopurine, a known and accepted antitumor agent, assayed concurrently with the test compounds, showed an inhibition of about 54% at a final concentration of 0.1 mg (0.6 μmole)/ml in this test system. Growth was determined turbidimetrically (3). Initially, the activity was determined at 1 mg/ml, in accord with the screening protocol (7), and the nine most active compounds were compared on an equimolar basis at 4.47 μmoles/ml, the final concentration. This concentration is equivalent to 1 mg of chloroacetyl-β-hydroxy-D-norleucine B/ml, the compound previously found to be the most active in these studies (2).

Of the 28 benzoyl compounds tested, the nine listed in Table I showed pronounced activity, greater than that

Table I—Effect of Equimolar Concentrations of *N*-Benzoyl Derivatives of Amino Acids and Amino Acid Analogs on the Growth of *L. casei* 7469 [a]

Compound	Inhibition[b], %
N-Benzoyl-*o*-fluoro-DL-phenylalanine	82
N-Benzoyl-*m*-fluoro-DL-phenylalanine	96
N-Benzoyl-*p*-fluoro-DL-phenylalanine	72
N-Benzoyl-β-2-thienyl-DL-alanine	76
N-Benzoyl-β-3-thienyl-DL-alanine	56
N^{α}-Benzoyl-L-tryptophan	74
N-Benzoyl-*p*-chloro-DL-phenylalanine	94
N^{α}-Benzoyl-*p*-nitro-L-phenylalanine	78
N^{α}-*p*-Nitrobenzoyl-L-phenylalanine	78

[a] Maximum growth in inoculated control tubes containing no test compound, measured turbidimetrically, was 166-173 Klett units. [b] Concentration was 4.47 μmoles/ml and was the final concentration in the assay system.

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observed for any acyl compounds previously tested. These compounds exhibited inhibition greater than 50% at 1 mg/ml, thus meeting the criterion of positivity set in the microbiological assay protocol (7). Therefore, these compounds have been submitted for antitumor test in mammalian systems.

Most of the active compounds were derivatives of phenylalanine analogs. Their activity was tested against melanomas, in which phenylalanine metabolism is believed to be intimately involved.

Detailed description of these findings will be reported elsewhere.

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Chlordiazepoxide: A New, More Sensitive and Specific Radioimmunoassay

Keyphrases ☐ Chlordiazepoxide—radioimmunoassay, in blood, plasma, saliva ☐ Radioimmunoassay—chlordiazepoxide in blood, plasma, saliva ☐ Tranquilizers—chlordiazepoxide, radioimmunoassay in blood, plasma, saliva

To the Editor:

In 1975, we reported (1) in detail the development of the first radioimmunoassay (RIA) for the widely used antianxiety agent chlordiazepoxide. Since that time, the radioimmunoassay has been used extensively here for studies involving the pharmacokinetic and biopharmaceutical evaluation of different formulations of the drug. Recently, however, we directed our attention toward measuring chlordiazepoxide in microsamples of whole blood, which can be simply obtained by a fingerstick, and in saliva, which offers a noninvasive approach. Simultaneous determination of chlordiazepoxide in blood/plasma and saliva might also allow us to evaluate both the pharmacokinetics and extent of *in vivo* drug protein binding as reflected by its concentrations in the two media. However, since the original radioimmunoassay procedure employed relatively low specific activity 2-^{14}C-chlordiazepoxide as the radioligand, it lacked the necessary sensitivity to achieve these aims.

We now wish to report the development of a radioimmunoassay for chlordiazepoxide that incorporates both a new radioligand and an antiserum.

The new hapten, 5-(4-aminophenyl)-7-chloro-2-methylamino-3*H*-1,4-benzodiazepine-4-oxide, was diazotized with 0.9 equivalents of nitrous acid and coupled to bovine serum albumin essentially as described by Peskar and Spector (2). The excess hapten was removed by exhaustive dialysis, and the conjugate was isolated by lyophilization as a brick-red powder. Rabbits were immunized with the conjugate as previously described (1), and the antiserum with the highest titer (1:15,000) of antibodies to chlordiazepoxide was used for all further studies.

The new radioligand, 8-^{3}H-chlordiazepoxide, was prepared by selective reduction of 2-amino-4,5-dichlorobenzophenone with tritium over Lindlar catalyst to yield 4-^{3}H-2-amino-5-chlorobenzophenone, which was converted to the desired product as previously described (3, 4). The 8-^{3}H-chlordiazepoxide was crystallized as its hydrochloride salt from methanol–ether to yield material with a specific activity of 17.8 Ci/mmole.

The radioimmunoassay procedure was identical with that recently described by Dixon and Crews (5) for diazepam. A logit–log calibration curve for chlordiazepoxide was linear between 50 and 5000 pg/tube, which represents a 40-fold increase in sensitivity over the original procedure (1). Routinely, 10 μl of blood or plasma was diluted to 1 ml with assay buffer and a 100-μl aliquot was taken for analysis. Saliva (100 μl) was assayed without dilution. Under these conditions, the working limits of sensitivity for blood/plasma and saliva were 50 and 0.5 ng/ml of chlordiazepoxide, respectively.

The new antiserum exhibited a fortuitously improved specificity toward chlordiazepoxide in that the cross-reactivity of a major metabolite, *N*-desmethylchlordiazepoxide, was <1% as opposed to 5% with the original antiserum (1). Other metabolites found in plasma, demoxepam and *N*-desmethyldiazepam, still cross-reacted less than 1%. A similar lack of cross-reactivity was found with all other benzodiazepines marketed in the United States. Amitriptyline and its metabolite, nortriptyline, showed no interference in the determination of chlordiazepoxide. The latter finding is particularly relevant in view of the recent introduction of a chlordiazepoxide–amitriptyline combination drug.

Preliminary studies using the new radioimmunoassay have indicated a chlordiazepoxide saliva to plasma concentration ratio of about 0.03, which is in close agreement with the ratio of unbound to bound chlordiazepoxide in plasma that we determined by equilibrium dialysis.

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