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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