lowing single oral doses of amitriptyline (25 mg) and amitriptyline (25 mg) plus diazepam (5 mg) were studied using the radioimmunoassay. Peak drug concentrations were reached 3.2 ± 0.9 h and 2.6 ± 0.7 h respectively after ingestion, while the peak concentrations of amitriptyline ranged from 25–38 ng/ml and between 18–47 ng/ml respectively.

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A simple radioimmunoassay for plasma diazepam and its application to single dose studies in man

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The value of monitoring antidepressant drug levels to rationalize therapy has been established (Montgomery, Braithwaite & Crammer, 1977). We have developed a sensitive radioimmunoassay for measuring plasma diazepam concentrations and used it to study the elimination of diazepam from the blood of four volunteers following a single (5 mg) oral dose of the drug.

Antibodies were raised in a rabbit against a diazepam–bovine serum albumin conjugate (Peskar & Spector, 1973; supplied by Roche Products). Immunogen (200 μg) was emulsified in Marcol 52/10% Arlacel A adjuvant (Robinson, Morris & Marks, 1975) and injected intradermally. Venous blood was collected after each booster and the serum lyophilized in 1 ml aliquots.

The assay was carried out in 0.1 M phosphate buffered saline pH 7.4, containing 0.1% gelatin and 0.1% sodium azide, with [^{3}H]-diazepam (s.a. 39 Ci/mmole; New England Nuclear) as the label. Using the antiserum at a final dilution of 1:1200 this assay has a sensitivity (Albano & Ekins, 1970) of 0.1 ng/ml diazepam. Plasma samples from patients receiving the drug routinely can be diluted 1:100 before being assayed, thus eliminating the necessity for extraction procedures prior to assay.

The avidity constant, Ka, of the antiserum, determined from a modified Scatchard plot, was 4.3098×10^9 l/mol at a binding site concentration of 0.439×10^{-9} mol/l. Cross-reactivity studies indicated that the antiserum was specific for diazepam, only flunitrazepam showing any interference in the assay (<10%). The major metabolite of diazepam, N-desmethyl-diazepam, did not cross-react with the antiserum. The mean intra-assay coefficient of variation was 3.62 ± 1.64% while the mean inter-assay coefficient of variation was 9.11 ± 2.85% (n = 10). A comparison of the radioimmunoassay with a gas–liquid chromatographic method in current use (Rutherford, 1977) demonstrated a good correlation between the two techniques (r = 0.984; n = 45).

Four volunteers received a single (5 mg) oral dose of diazepam and venous blood was sampled over a 24 h period. Determination of plasma levels showed that peak concentrations were reached between 0.5 and 1.5 h after administration. The peak levels ranged from 64 to 160 ng/ml. These values demonstrate individual variation in diazepam absorption and metabolism and may be used to predict steady-state levels during prolonged administration of the drug.

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