with those communicated previously (Caldwell, Lancaster, Monks & Smith, 1977).

[^{14}C]-Aminophylline injection was prepared from 8-[^{14}C]-theophylline and ethylenediamine as described in the British Pharmaceutical Codex (1963), sterilized by ultrafiltration and administered to three male volunteers by intravenous injection (125 mg; 10 μCi). The subjects kept their normal diets, and collection of urine samples, determination of urinary metabolites and pharmacokinetic analysis was performed as previously described (Caldwell *et al.*, 1977).

The metabolism of aminophylline was the same as that of theophylline; the excretion products being 3-methylxanthine, 1,3-dimethyluric acid and 1-methyluric acid in addition to theophylline and the pharmacokinetic model describing the disposition of aminophylline was identical with that previously described for theophylline (Caldwell *et al.*, 1977). However, the recovery of [^{14}C] in the 0–24 h urine was higher for aminophylline (theophylline, 76.3 ± 6.9% (mean ± s.d.); aminophylline, 87.0 ± 2.0; $P < 0.05$), and this was accompanied by significant increases in the first-order rate constant for 1,3-dimethyluric acid elimination (K_{el}^{DMU}, theophylline, 0.022 h^{-1}; aminophylline, 0.034 h^{-1}; $P < 0.05$) and in the V_{max} of the Michaelis–Menten expression for 3-methylxanthine elimination (V_{max}^{3MX}, theophylline, 0.94 mg h^{-1}; aminophylline, 1.66 mg h^{-1}; $P < 0.05$). The elimination $T_{1/2}$ of total urine [^{14}C] was reduced from 10.0 ± 2.8 h for theophylline to 7.4 ± 0.4 h for aminophylline ($P < 0.05$).

It is clear that there are significant differences between the metabolism and pharmacokinetics of aminophylline and theophylline in man. Thus, the complexation of theophylline and ethylenediamine alters its disposition when compared with theophylline alone, with increases in the rate and extent of conversion to, and elimination of, 1,3-dimethyluric acid and 3-methylxanthine. These increases account for the greater urinary excretion of [^{14}C] after aminophylline compared with theophylline. The reasons for this difference in behaviour between the two drugs is not clear, since aminophylline is commonly regarded as the ethylenediamine salt of theophylline.

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A radioimmunoassay for amitriptyline and nortriptyline

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Routine plasma level determinations are becoming increasingly important in antidepressant therapy (Montgomery, Braithwaite & Crammer, 1977). A sensitive radioimmunoassay for amitriptyline and nortriptyline, suitable for routine use, has been developed which requires no sample preparation other than a five-fold dilution of the plasma.

Using [^{3}H]-amitriptyline (sp.act. = 10.0 Ci/mmole) as the label the antiserum, raised in a sheep (Aherne, Piall & Marks, 1976) could be used at a final dilution of 1:1400. The antiserum avidity constant was 9.3×10^{7} l/mol and the assay sensitivity was 0.86 ng/ml.

Cross-reactivity studies demonstrated that the assay was specific for amitriptyline and nortriptyline, showing no cross-reactivity with their metabolites or any drugs likely to be given in combined therapy. The mean intra- and inter-assay coefficients of variation were 4.7% and 9.8% ($n = 10$) respectively.

Comparison of the assay with a routine gas–liquid chromatographic method for nortriptyline was made on plasma samples collected from three patient groups. For a controlled in-patient group the correlation coefficient, r, for the two methods was 0.9799 ($n = 83$), for an uncontrolled out-patient group r = 0.9528 ($n = 45$) and for a third group, receiving single doses prior to therapy, r = 0.9438 ($n = 25$).

Plasma amitriptyline levels in five volunteers fol-

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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