Tissue distribution of radioactivity after injection of [^{14}C]nitrazepam in young and old rats

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Both in patients and healthy volunteers old age is associated with an increased sensitivity to the hypnotic nitrazepam (Evans & Jarvis, 1972; Castleden, George & others, 1977). Plasma concentration data indicate that this is partly due to altered pharmacokinetics in aged patients (Iisalo, Kangas & Ruikka, 1977) but not in healthy aged subjects (Castleden & others, 1977). To examine the problem of age-related increased nitrazepam sensitivity further, we decided to measure the concentrations of radioactivity in the brain and other tissues of young and old rats at various times after a single dose of [^{14}C]nitrazepam. An additional purpose of this work was to supplement the limited information available on the tissue distribution of nitrazepam, concentrations only having been previously determined in blood, brain and liver (Tanayama, Momose & Kanai, 1974; Yanagi, Haga & others, 1975).

"Young" (100 days old) and "old" (540 days old) male Wistar rats were injected with 5[^{14}C]nitrazepam 40 mg kg^{-1} (4·2 μCi kg^{-1}), intraperitoneally to avoid any possible age related variation in drug absorption from the gastrointestinal tract, the drug being dissolved in dimethylsulphoxide (40 mg ml^{-1}) which was chosen because it appears to have no effect on the onset or duration of action of centrally acting drugs (see e.g. Dixon, Adamson & others, 1965). The rats were killed at 2, 3·3, 4·7 and 6 h after injection and blood and tissue (brain, spleen, kidney, liver, heart, lung and small intestine) samples were taken for measurement of radioactivity by liquid scintillation spectrometry. For this aqueous tissue homogenates (25% w/v) were prepared and 0·25–0·5 ml samples were digested (heated for 1 h at 60° with 1 ml Soluene, Packard Instrument Co., Inc. Illinois) and decolourized (0·2 ml isopropanol followed by 0·2 ml 30–35% hydrogen peroxide) in counting vials before the addition of 10 ml of a toluene-based scintillator (NE 260, Nuclear Enterprises Ltd., Edinburgh) to each. Erythrocytes were diluted with an equal volume of water and 0·2 ml aliquots treated similarly to the tissue samples except that 0·5 ml Protosol (33% v/v in ethanol) and 15 ml Biofluor (New England Nuclear, Boston, Mass.) were used as the solubilizer and scintillator respectively and 0·5 ml of 0·5 M hydrochloric acid solution was added to each vial before counting.

In a few young rats [^{14}C]nitrazepam of a higher specific activity (40 mg kg^{-1}, 20 μCi kg^{-1}) was injected to facilitate drug/metabolite analysis. Where the drug of higher specific activity had been used the rats were killed after 3 h and homogenates of brain and liver were made alkaline and extracted with butan-1-ol (95% of radioactivity extracted) and the extracts analysed by thin-layer chromatography (Tanayama & others, 1974; Yanagi & others, 1975).

Nitrazepam plasma half-lives were calculated from a least squares regression analysis of logarithm of plasma nitrazepam concentration *vs* time plots. Apparent volume of distribution (Vd) was calculated from the single-compartment formula Vd = dose/Co where Co is the theoretical plasma nitrazepam concentration at the time of injection. Plasma clearance (1 litre h^{-1} kg^{-1}) was calculated by dividing Vd (1 litre kg^{-1}) by half-life (h) and multiplying by 0·693.

Despite the old rats being visibly more sedated by the 40 mg kg^{-1} dose of [^{14}C]nitrazepam the plasma concentrations of radioactivity at each of the four times measured were similar in young and old animals (Fig. 1). The corresponding pharmacokinetic parameters in

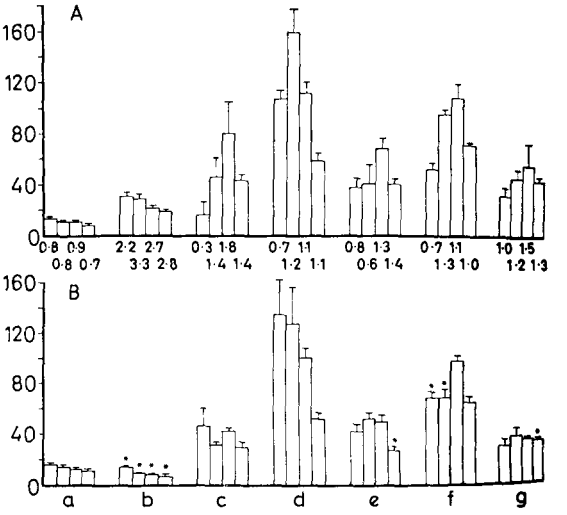

FIG. 1. The tissue distribution of radioactivity after [^{14}C]nitrazepam injection to A: old and B: young rats. Young (100 day) or old (540 day) male Wistar rats were injected intraperitoneally with 5[^{14}C]nitrazepam (40 mg kg^{-1} 4·2 μCi kg^{-1}). For each block of four columns, tissue sampling times were (left to right) 2, 3·3, 4·7 and 6 h. Each column represents the mean ± s.e. from 3 rats. The figures associated with the columns are the respective old : young tissue radioactivity concentration ratios at each sampling time. The asterisks indicate statistically significant differences between young and old rats (Student's *t*-test). a: Plasma; b: brain; c: spleen; d: kidney; e: heart; f: liver; g: lung. Ordinate: Tissue radioactivity (μg nitrazepam equivalent g^{-1} or ml^{-1}).

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young and old rats respectively were plasma clearance, 0·27 and 0·29 litre h^{-1}; plasma half-life, 5·4 and 5·6 h; apparent volume of distribution, 2·1 and 2·4 litre kg^{-1}. In contrast the concentrations of radioactivity in the brain were consistently and significantly two or three times greater in the old rats (Fig. 1). There was no consistent difference of this magnitude between young and old animals with the other organs examined; the mean old: young tissue radioactivity concentration ratio in the spleen, kidney, heart, liver and lung was 1·1.

The highest concentrations of radioactivity were found in the kidney and liver, followed by the spleen, heart and lung, with the lowest values in the plasma and brain (Fig. 1). High concentrations of radioactivity (similar to those found in the liver and kidney) were also measured in the wall of the small intestine (data not presented) although this could have been a direct result of injecting the drug intraperitoneally. Peak concentrations of radioactivity were achieved in plasma and brain by 2 h,but in some other tissues, and particularly in the old rats, peaks tended to occur later (Fig. 1). Over the period of the experiment the erythrocyte/plasma radioactivity concentration ratio was 0·6–0·7.

In the brain or liver of the young rats killed at 3 h at least 85% of the radioactivity could be identified chromatographically as unchanged nitrazepam. The remaining radioactivity on the chromatograms appeared to be "background" and did not correspond clearly to any particular chromatographic locus.

Our results in young rats essentially agree with those of previous workers using [^{14}C]nitrazepam in rats (Tanayama & others, 1974; Yanagi & others, 1975) in that the brain: plasma radioactivity concentration ratio was about 0·7 and that the liver concentrations of radioactivity were markedly higher than in the plasma and brain. The higher concentration of [^{14}C]nitrazepam-derived radioactivity in the brain of old rats was surprising and it is possible that an analogous distribution change in man could account, wholly or in part, for the increased sensitivity to nitrazepam seen in elderly subjects and patients.

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Ethanol and the disposition of amylobarbitone: effect of dose and significance as a mechanism for increased toxicity

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Although there is a general acceptance that the clinical toxicity of barbiturate hypnotics is increased by concomitant ingestion of ethanol (Patel, Roy & Wilson, 1972) the results of animal experiments conflict about whether the interaction is one of synergism (Wiberg, Coldwell & Trenholm, 1969), addition (Smith & Hexheimer, 1969) or indeed of antagonism (Curry & Scales, 1973). Considering only those studies which indicate that ethanol increases the toxicity of barbiturates, there remains disagreement about the mechanism. In particular, it is not certain to what extent impairment of drug metabolism by ethanol, with a consequent prolongation of half-life of the barbiturate, is the cause of the increased toxicity (Wiberg & others, 1969; Schuppel, 1972).

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Schuppel has reviewed the results of his group's experiments on the *in vitro* effects of ethanol on microsomal mixed function oxidase activity and on the *in vivo* elimination of a number of drugs in the rat when ethanol is co-administered, and concludes that inhibition of barbiturate metabolism due to ethanol is the cause of the increased toxicity of barbiturates which they have observed (Schuppel, 1972).

We have examined the effects of hypnotic and non-hypnotic doses of ethanol on the effects, distribution and elimination of amylobarbitone in adult male Wistar rats. The drugs were given as intraperitoneal injections of aqueous solutions. The plasma disappearance of amylobarbitone was measured by collecting blood from groups of 4 rats decapitated at various intervals between 20 and 300 min after injection of 100 mg kg^{-1} amylobarbitone sodium, or of 100 mg kg^{-1} amylobar-