and a is the volume collected from the 3rd–13th min after the ADH injection.

The antidiuretic potency of ADH is shown in Fig. 1. Values of antidiuretic effect obtained from 10 untreated and 10 propranolol-treated rats are expressed in terms of dose of ADH. Each point is the mean of 6 determinations at each dose level of ADH administered at 1 h intervals, and shows that ADH produces a similar degree of antidiuresis in different animals and that the antidiuretic response increased linearly with the logarithm of the dose.

The untreated and propranolol-treated rats exhibited a similar sensitivity to ADH, but the log dose response regression line of the propranolol-treated animals was more shallow than that for the untreated rats, and is due to the quicker recovery of diuretic activity from the effects of ADH.

Dicker (1953) found that tachyphylaxis developed to low concentrations of ADH if the time interval between doses was less than 45–60 min. Table 1 summarises results which confirm this finding, tachyphylaxis being observed with doses of ADH as low as 2·5 μU/100 g when given at 30 min intervals. Propranolol (0·1 mg kg^{-1}) treatment allowed the estimation of low concentrations of ADH (2·5–10·0 μU/100 g) at 30 min intervals, but tachyphylaxis was seen with higher doses.

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Some observations on the attempted separation of isoenzymes of monoamine oxidase

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Studies of the varying activity of monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating) EC 1.4.3.4.] isolated from different species and tissues towards various substrates (Alles & Heegaard, 1943) inhibitors (Hardegg & Heilbron, 1961; Johnston, 1968; Hall, Logan & Parsons, 1969) and heat sensitivity (Youdim & Sourkes, 1965) have suggested that MAO exists in several forms or may be represented as a family of related enzymes. These suggestions have been strengthened by the frequent demonstration of multiple bands of enzymatic activity on gel electrophoresis of solubilized enzyme preparations (Youdim & Sandler, 1967; Kim & D'Iorio, 1968). The number of such bands detected however, varies with the tissue and species employed in the isolation (for review see Sandler & Youdim, 1972).

The present work was initiated as a study of the potential substrate specificity or selectivity of the various separable isoenzymic forms of MAO. Since that time however, more recent studies have raised doubts regarding the specificity of the detection methods employed for the colourimetric detection of the enzyme in polyacrylamide discs (Diaz Borges & D'Iorio, 1973) and the methods employed in isolating the enzyme (Houslay & Tipton, 1973) suggesting that some of these bands of activity may be artifacts. Our own results, reported here, are in agreement with the more recent studies, demonstrating a lack of specificity in the detection procedure. In addition, it is suggested that the presence of non-ionic surfactants employed in the solubilization of the enzyme e.g. Triton X-100 may influence the behaviour of the enzyme preparation on gel filtration.

The enzyme MAO was isolated from the liver and brain of male Sprague-Dawley rats (250–300 g) by previously reported methods (Youdim & Collins, 1971) with only slight modification. Thus, the enzyme preparation, after solubilization in Triton X-100 (1·5% w/v) in phosphate buffer (0·05 M; pH 7·4), was precipitated between 25 and 55% ammonium sulphate saturation and the product dialysed, after redissolving in phosphate buffer (0·05 M; pH 7·4), against a total of 500 volumes of buffer for up to 60 h. The effect of dialysis time upon the subsequent elution of the enzyme

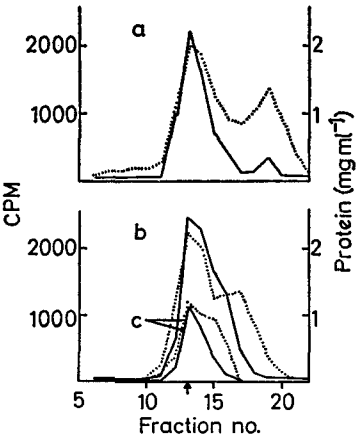

FIG. 1. Effect of dialysis time upon gel filtration behaviour of rat MAO. (a) Liver enzyme preparation dialysed 24–48 h. (b) Liver enzyme preparation dialysed 60 h. (c) Brain enzyme preparation dialysed 60 h. Solid line—enzyme activity, substrate tyramine. Dotted line—protein concentration. Arrow indicates void volume (105 ml).

* Correspondence.

from a column of Sephadex G-200 (enzyme activity was monitored using ^{14}C-labelled tryptamine) is shown in Fig. 1. Although most of the enzyme activity is eluted from the column in the void volume (mol. wt > 60 0000), dialysis of the enzyme preparation for less than approximately 60 h resulted in a small amount of enzymic activity being retained by the column. To investigate the possibility that this phenomenon could be due to the high affinity of the protein for the non-ionic surfactant and also to stabilization of the enzyme in the deaggregated form, Triton X-100 was added to enzyme which had been previously dialysed for 60 h and which was eluted in the void volume. In the presence of 0·3% of Triton X-100, this enzyme preparation was completely retarded when subjected to gel filtration on a column of Sephadex G-200. Although Triton X-100 is frequently employed in the initial solubilization of the enzyme, dialysis of the partially purified enzyme after ammonium sulphate precipitation is rarely carried out and the enzyme which shows variable behaviour upon gel filtration, appears as a single active fraction of molecular weight approximately 300 000 (Youdim & Collins, 1971; Tipton, Youdim & Spires, 1972) or in two active fractions, one of which is eluted in the void volume of the column (Shih & Eiduson, 1973; Carper, Stoddard & Martin, 1974). Oreland (1972) prepared a pig liver MAO preparation without the use of detergents which on Sephadex G-200 showed a single peak of activity appearing in the void volume. Addition of sodium cholate to the enzyme preparation in increasing amounts resulted in the retarding by the column, of increasing quantities of the enzyme, the retarded fraction in the presence of 5–10% of sodium cholate separated into two fractions of molecular weight 150 000 and 290 000 daltons approximately. Thus, it would seem that the variable gel filtration behaviour of MAO reported by different workers can be partially attributed to the differing amounts of ionic or non-ionic detergent remaining in the enzyme preparation at that stage.

In addition to the already mentioned variations, in the molecular weight of the MAO, much variation has been reported in the number of isoenzymes detected on polyacrylamide gel electrophoresis (for review see Sandler & Youdim, 1972). We therefore made electrophoretic separations of the enzyme at various stages of the isolation, enzymatic activity being monitored by the colorimetric method of Glenner, Burtner & Brown (1957) and also by the use of ^{14}C-labelled substrates on portions of the segmented gel (Wurtman & Axelrod, 1963). In the pre-column enzyme preparation (the dialysed enzyme preparation before gel filtration) although four staining bands were detected in the gel (corresponding in the unstained gel to two slightly turbid bands and two narrow refractive bands of greater mobility) only the least mobile band showed enzyme activity when tested using ^{14}C-labelled substrates. The results in both the nitrobluetetrazolium staining test and the radiolabelled substrate test were independent of the structure of the substrate employed (tryptamine, tyramine, or β-phenethylamine) and tissue source of the enzyme (brain or liver).

Using the post-column enzyme preparation (enzyme preparation after gel filtration and appearing in the void volume) two bands (a single turbid and a single narrow refractive band) could be seen in the unstained gel, but only one of these (the more mobile refractive band) consistently stained in the nitrobluetetrazolium colour reaction whereas only the non-staining turbid band showed activity when tested by the radio chemical method. These results further demonstrate the inadequacy of the nitrobluetetrazolium staining method in studies of the enzyme monoamine oxidase (Diaz Borges & D'Iorio, 1973; Guha & Ghosh, 1970).

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