between the effects of the drugs on leucocyte migration and prostaglandin accumulation in the sponge model, in accord with the observation that prostaglandins are not chemotactic in the rat or man (Walker & others, 1976b). It must be concluded that acidic non-steroidal anti-inflammatory drugs of this class do not have a single site of action but exert at least two independent anti-inflammatory effects.

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2-Dimethylaminoethanol (Deaner) in body fluids

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2-Dimethylaminoethanol (DMAE) which was suggested to act as a central stimulant (Pfeiffer, Jenney & others, 1957), has been used clinically as an alternative to amphetamine in the treatment of behavioural disorders in children with the minimal brain dysfunction syndrome (Oettinger, 1958; Lewis & Young, 1975). The pharmacological properties of DMAE are, however, incompletely known partly because no method for its assay has yet been described. In this communication we describe two techniques for the determination of DMAE in body fluids both of which are based on gas-liquid chromatographic (g.l.c.) separation of DMAE following derivatization with propionyl chloride. The derived DMAE is quantified either by flame ionization or by mass fragmentography (m.f.). The methods are applied in the determination of DMAE in body fluids of rabbits and humans kept on the drug in pharmacological doses.

DMAE assay I. 0·3 ml 20% trichloroacetic acid were added to 0·5 ml plasma or csf. The precipitate formed was centrifuged, the clear supernatant extracted three times with 5 ml ether, and then dried under nitrogen. Shaking for 10 min with 0·5 ml methanol extracted the residue and after centrifugation the clear supernatant was again dried under nitrogen. The residue was then extracted with 1·2 ml of chloroform–5 M sodium hydroxide (5 : 1) by shaking for 15 min followed by centrifugation. To 0·75 ml of the chloroform phase was added 50 μl redistilled propionyl chloride and after 10 min at room temperature (22°) the solution was dried under nitrogen. The residue was redissolved in 25 μl chloroform containing DMAE-butanoate* as an internal standard at a concentration to match approximately the concentration of the DMAE to be assayed (5–250 nmol ml^{-1}). 1–3 μl of the chloroform extract were injected into a Pye Unicam GCV gas chromatograph. The compounds were separated on a glass column (1 m × 2 mm i.d.) packed with Pennwalt 223 amine packing (80–100 mesh, Applied Science Lab), at 170°. Carrier gas

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* 2-Dimethylaminoethylbutanoate (DMAE-butanoate) was prepared from distilled DMAE and butanoyl chloride which were mixed in equimolar concentrations in ether at 0°. The precipitate formed was washed several times with ether and then dried in vacuum at room temperature.

nitrogen 30 ml min^{-1}. Injection port and flame detector temperature were 230° and 225°. The concentration of DMAE was calculated from the peak height ratio DMAE-propanoate/DMAE-butanoate × concentration of DMAE-butanoate added. To evaluate the total recovery of DMAE 24 nmol 1,2-^{14}C-DMAE (spec. act. 5 Ci mol^{-1}; New England Nuclear) were added to 0·5 ml rabbit plasma, and processed as described. 85·8 ± 3·3% (mean ± s.e.m., n = 8) of the total radioactivity was recovered in the final chloroform extract. A standard curve was calculated by measuring the peak height ratios of DMAE-propanoate/DMAE-butanoate *versus* seven different concentrations of DMAE in rabbit plasma. A linear relation was found in the range of 12–250 μM DMAE as expressed by the equation h = 0·0095 × [DMAE] + 0·0160; (r = 0·9969) h is the measured ratio value normalized at a concentration of 100 μM DMAE-butanoate and corrected for a DMAE recovery of 86%. [DMAE] is the concentration of DMAE added to plasma. To assess the precision of the method replicate assays on a pooled sample of plasma were made, resulting in 8·4 ± 0·5 nmol DMAE (mean ± s.e.m., n = 7).

DMAE assay II. 0·5 nmol [$^2H_4$] DMAE† in 50 μl were added as an internal standard to 0·5 ml plasma or csf. The procedure was then carried out as described above up to and including the step of drying the propionylates products. The residue was then redissolved in 25 μl chloroform. 1-3 μl were injected into a g.c./m.f. (LKB 2091) helium replacing nitrogen as carrier gas. The multiple ion detector of the m.f. was focussed on *m/e* values of 58 and 60. *m/e* 58 is the base peak of DMAE-esters and *m/e* 60 that of [$^2H_4$] DMAE-esters (Karlén & others, 1974). The concentration of DMAE was calculated from the peak height ratio *m/e* 58/*m/e* 60 multiplied by the concentration of [$^2H_4$] DMAE. The standard curve for DMAE measured at eight different concentrations in the range of 0·1–4·0 μM is expressed by the equation DMAE/[$^2H_4$] DMAE = 1·1480 × [DMAE] + 0·1437; (r = 0·9971). As seen the ratio DMAE/[$^2H_4$] DMAE is linearly related to the DMAE concentration.

Applications. Chinchilla male rabbits (1·8–2·0 kg) were administered 15 or 30 mM DMAE (the base of DMAE neutralized with HCl) in the drinking water and 2–3 ml blood from an ear vein was collected in heparinized tubes at different times. 0·5–1·2 ml blood-free csf was drawn by puncture of cisterna magna with a 23-gauge needle 5 min after injection of 40–50 mg kg^{-1} (i.v.) of pentobarbitone. When kept on a daily DMAE dose of 1·8 ± 0·2 mmol per animal, the plasma DMAE reached a concentration of 6–7 μM (5·3 ± 1·0 μM after 3 days; 6·9 ± 0·9 μM after 7 days) and at a dose of 4·5 ± 0·3 mmol it reached 12–18 μM (17·3 ± 1·5 μM after 3 days; 18·6 ± 3·5 μM after 7 days; 11·6 ± 1·9 μM after 14 days). 36 h after withdrawal of DMAE no measurable amounts of the drug could be detected in plasma. The DMAE concentration in plasma (7·5 ± 0·8 μM) did not differ from that in csf (8·8 ± 1·0 μM) indicating that DMAE penetrates the blood/brain barrier. All data are means ± s.e.m. of five animals.

Nine psychiatric patients of either sex, ages 23–66, admitted to hospital for chronic anxiety, were given increasing daily doses of Deaner (*p*-acetylamidobenzoic acid salt of DMAE, generously supplied by Riker Laboratories Inc.) by mouth starting with 1·1 to reach 1·9 mmol (500 mg) after three days and 3·8 mmol (1000 mg) after one week. Vein blood was drawn after one week on each dose and 12 h after the last dose had been given. Patients thus kept on a daily oral dose of 1·9 mmol for one week yielded a plasma concentration of DMAE at 0·25 ± 0·04 μM (mean ± s.e.m., n = 6). When on 3·8 mmol Deaner, plasma DMAE reached 0·52 ± 0·14 μM (mean ± s.e.m., n = 7).

The sensitivity of the g.c. method allows assay of DMAE at pharmacological doses in plasma and csf of animals (Pfeiffer & others, 1957; Ceder & Schuberth, to be published). Clincially DMAE is given in much lower doses than to animals. For this reason the g.c./m.f. method was developed. The factor limiting the sensitivity of this method at about 0·05 μM DMAE is that the mass spectrum from [$^2H_4$] DMAE contains a fragment at *m/e* 58 with significant relative abundance (7–9%). Therefore, the standard curve does not pass through the origin. The reason for this apparent contamination of [$^2H_4$] DMAE is not known. However, preformed [$^2H_4$] DMAE-propionate contains no measurable fragment at *m/e* 58. The 'contamination' may therefore be due to an isotope exchange reaction occurring during the preparation of the extracts to be injected into the g.c./m.f. But the method is suitable for the assay of DMAE in plasma of patients on therapeutic doses of Deaner.

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† [$^2H_4$] DMAE was prepared by reduction of *N,N*-dimethylglycolamide with $LiAl_2H_4$ according to Karlén, Lundgren & others (1974).

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