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Separate anti-inflammatory effects of indomethacin, flurbiprofen and benoxaprofen

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An important query about non-steroidal anti-inflammatory drugs is whether they have either a common single mode of action or different multiple interactions with various aspects of inflammatory responses. An inhibitory action on prostaglandin biosynthesis has been advanced as a mechanism for the first possibility (Vane, 1973). More recently evidence has accumulated that the drugs possess anti-inflammatory effects which appear to be independent of prostaglandin systems (Bonta, Bult & others, 1977; Crook, Collins & others, 1976). One of the most relevant is an interference with the emigration of leucocytes into inflammatory sites (Walker, Smith & Ford-Hutchinson, 1976a). In the present work the effects of three acidic non-steroidal drugs, indomethacin, flurbiprofen and benoxaprofen, on the production of prostaglandins and the migration of leucocytes into an inflammatory exudate *in vivo*, have been studied. All three drugs possess a similar spectrum of experimental anti-inflammatory activity (Glenn, Rohloff & others, 1973; Adams, McCullough & Nicholson, 1975; Cashin, Dawson & Kitchen, 1977) but differ in that the first two are potent inhibitors of prostaglandin synthetase activity *in vitro* (Crook & Collins, 1975) whereas benoxaprofen is only a weak inhibitor of the enzyme system (Cashin & others, 1977).

Indomethacin was obtained from Merck Sharp and Dohme. Benoxaprofen (2-[4-chlorophenyl]-α-methyl-5-benzoxazone acetic acid) was obtained from Dr W. Dawson, Lilly Research Centre, Ltd, Windlesham, Surrey, England, and flurbiprofen (2-[fluoro-4-biphenylyl] propionic acid) was obtained from Dr S. Adams, Boots Drug Co. Ltd, Nottingham, England. The 9 h sponge implantation technique, the estimation of leucocyte migration and prostaglandin-like activity in the sponges were as described previously (Walker & others, 1976a). Drugs were administered orally as a suspension in Tween 80 to groups, each of 5 rats, 1 h before sponge implantation.

The effects of the three drugs on prostaglandin accumulation and leucocyte migration into the 9 h sponge exudates are shown in Table 1. Indomethacin and flurbiprofen, which are potent inhibitors of prostaglandin synthetase *in vitro*, strongly inhibited the accumulation of prostaglandin activity *in vivo* in the sponges. This inhibition occurred at lower dosages than those required to inhibit white cell migration in the system and well below those required to inhibit other experimental models of inflammation (Glenn & others, 1973; Adams & others, 1975). Benoxaprofen only showed inhibition of prostaglandin production *in vivo* at doses equivalent to those required to inhibit white cell migration in the model and similar to those needed to affect other experimental models (Cashin & others, 1977). These findings demonstrate a lack of correlation

Table 1. *Effects of systemic administration of indomethacin, benoxaprofen and flurbiprofen in prostaglandin content (PG) and leucocyte migration (LM) in 9 h sponge exudates in the rat.*

Dosage mg kg^{-1}	Mean inhibition (% of control values†) Indomethacin		Benoxaprofen		Flurbiprofen	
	PG	LM	PG	LM	PG	LM
0·1	35	0	—	—	69*	28
0·3	53*	0	—	—	81*	52*
1·0	93*	18*	36*	26	85*	72*
3·0	97*	50*	68*	40*	96*	54*
9·0	98*	73*	82*	68*	—	—

* $P < 0.05$ from results of corresponding control group.
† In the sponge exudates of the corresponding control group, the mean prostaglandin-like content was 22 ± 8 ng ml^{-1} and the total leucocyte count was 658 ± 163 × 10^4 ml^{-1} (15 rats).

* Correspondence.

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

* Correspondence

* 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.