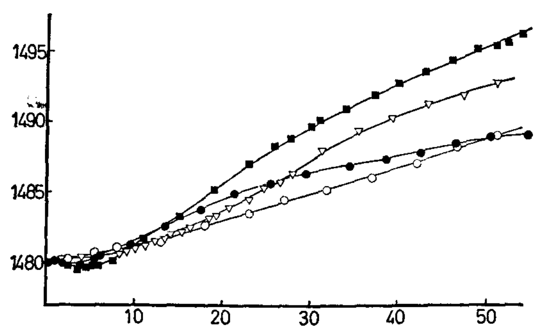

FIG. 1. Variation of sound velocity, U, with time during the diffusion of ●, sodium chloride; sodium salicylate; ■, mepyramine maleate and ○, carbachol into 3·5% gelatin gels. Ordinate: U (m s^{-1}). Abscissa: time (h).

inside the well-defined sound path in the gel rod can be obtained. Table 1 shows some preliminary results. For sodium chloride the results have been checked by means of chloride titrations carried out on samples taken from the appropriate gel volumes. Thus the technique here described may be a useful tool for fast and accurate monitoring of drug diffusion inside a gel rod.

To obtain diffusion coefficients from sound velocity measurements a new cell is being designed in which the sound velocity can be monitored at two positions of accurately known separation within the gel. The concentration and concentration gradient can thus be measured at both positions simultaneously, generating the necessary data from which diffusion coefficients may be calculated.

This preliminary report shows that measurements of the sound velocity as a function of time provides a new and non-destructive method of studying diffusion of drugs through polymer networks. It is proposed that the experimental technique here desciibed will be used in

Table 1. The drug concentration expressed as mol dm^{-3} in the gel volume through which the sound passes.

Time (h)	Sodium chloride	Sodium salicylate	Mepyramine maleate	Carbachol
6	0·023	0·016	0·019	0·009
12	0·054	0·033	0·020	0·029
18	0·082	0·049	0·029	0·076
24	0·111	0·073	0·039	0·090
30	0·121	0·097	0·049	0·113
36	0·132	0·120	0·059	0·129
42	0·144	0·135	0·068	0·132

the investigation of the effect of increased cross-linkage on drug release rate and in the determination of the drug release characteristics of polymer networks more suitable for use as implants.

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Inhibition of rabbit PMN lipoxygenase activity by benoxaprofen

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A variety of arachidonate lipoxygenase products exhibit both chemokinetic and chemotactic activity for polymorphonuclear cells (PMNs) (Samuelsson 1979). Recent studies using human PMN homogenates as a source of lipoxygenase enzyme(s) have shown that 5-hydroxyeicosatetraenoic acid (5-HETE) possesses the highest chemotactic activity of the monohydroxy acids detected (Goetzl & Sun 1979). The authors suggest that endogenous formation and release of these compounds from intact cells upon suitable stimulation may be involved in the control of cell movement into inflammatory exudates.

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Vane (1971) proposed that the common mechanism of action of non-steroidal acidic anti-inflammatory compounds (NSAI) was the inhibition of the enzyme complex prostaglandin synthetase (cyclooxygenase). Many supportive data have been adduced since that time (Flower 1974; Ferreira & Vane 1979) but a number of workers have felt that the story was incomplete (Smith 1978; Dawson 1979a). The recent work of Higgs et al (1979) suggesting a link between cell migration and the lipoxygenase products of arachidonate may well provide a further insight into the mechanism of action of NSAI.

Benoxaprofen, a new anti-rheumatic/anti-inflammatory agent, exhibits low arachidonic cyclooxygenase

activity but is comparable to indomethacin in experimental models of inflammation (Ford-Hutchinson et al 1977; Cashin et al 1977). The drug appears to modify inflammatory cell function, in particular that of the monocyte/macrophage by an unknown mechanism. (Meacock & Kitchen 1979).

This communication describes the effect of benoxaprofen on the generation of lipoxygenase products using a modification of the method of Borgeat & Samuelsson (1979). Intact rabbit PMNs were the source of lipoxygenase activity.

Rabbit peritoneal PMNs were elicited with glycogen (100 ml, 0·1%, 4 h) washed and resuspended in buffered Eagle's minimum essential medium (MEM) pH 7·3 at a concentration of 6×10^7 cells ml^{-1}. Samples (0·5 ml) were prewarmed to 37 °C for 15 min followed by a further incubation for 10 min with 0·5 ml of drug solutions prepared in MEM.

20 μl of ionophore (A23187, 0·5 μg) in DMSO and [1-^{14}C]arachidonic acid (0·7 ng) (55·5 mCi $mmol^{-1}$, Radiochemical Centre, Amersham) in ethanol–MEM (1:9 v/v) were added simultaneously. The incubations were terminated after 5 min by addition of 10 volumes of methanol. Methanol was removed under reduced pressure and the acidic lipids recovered by acidification (pH 3·0) using citric acid and extraction with 2 × 15 ml diethyl ether. Samples were reduced to dryness, redissolved in a small volume of ether, applied to silica gel t.l.c. plates and developed with a hexane–ether–acetic acid (50:50:3 v/v) mixture. Radioactivity was detected by radioscanning and quantitated by a standard liquid scintillation counting technique.

In one experiment, a bulk incubation (10 ml) was carried out to which cold arachidonic acid was also added (100 μg). Extracted material was subjected to reverse-phase h.p.l.c. and the u.v. absorbing peaks containing radiolabel were run on t.l.c. Their chemical composition was determined by derivatization and g.c.-m.s. analysis.

Benoxaprofen at concentrations of 10–50 $\mu g\ ml^{-1}$ produced a dose-related inhibition of the two main lipoxygenase product peaks (Table 1). Peak A has been tentatively identified as a mixture of 5-, 9- and 11-HETEs. Peak B has been positively identified as 5,12-diHETE (D. J. Osborne, unpublished observations). Indomethacin at 1 $\mu g\ ml^{-1}$, caused a slight potentiation of both major peaks. Indomethacin, 3 and 0·3 $\mu g\ ml^{-1}$, was also tested in one experiment and had no effect on the formation of lipoxygenase products.

The inhibition of arachidonic lipoxygenase by benoxaprofen provides a new facet to the pharmacology of this compound. Meacock & Kitchen (1979) have shown that benoxaprofen inhibits the accumulation of cells in inflammatory exudates and it is possible that this is a reflection of lipoxygenase inhibition at the site of inflammation. However, benoxaprofen also inhibits the chemotactic response of monocytes to zymosan activated serum (ZAS), a reaction presumably due to the

Table 1. Inhibition of rabbit polymorphonuclear leucocyte lipoxygenase activity by benoxaprofen. Incubation mixture (1 ml) contains: 3×10^7 cells 0·7 ng [1-^{14}C]arachidonic acid in Eagles medium with 0·5 μg ionophore (A23187) (challenged control, CC) or without ionophore (unchallenged control, UC). Compounds were used at the concentrations stated. Peaks are quantitated as % of total radioactivity. The R_F of Peak A (monohydroxy acids) was 0·38 ± 0·04 and of Peak B (5,12-diHETE) was 0·08 ± 0·008 in the hexane–ether–acetic acid (50:50:3) system. n = at least 8 for each group.

Drug concn. ($\mu g\ ml^{-1}$)	Peak A Mean ± s.e.m.	% Change (−UC)	Peak B Mean ± s.e.m.	% Change (−UC)
UC	4·0 ± 1·2	—	4·5 ± 0·6	—
CC	15·7 ± 1·1	—	25·6 ± 1·5	—
Benoxaprofen				
50	2·9 ± 0·4***	100 ↓	2·9 ± 1·5***	100 ↓
30	7·6 ± 1·6***	69·2 ↓	10·1 ± 2·6***	73·5 ↓
20	12·4 ± 0·9**	28·2 ↓	16·7 ± 1·9**	42·8 ↓
10	17·5 ± 2·0	13·3 ↑	24·0 ± 1·1	7·6 ↓
Indomethacin				
1	20·5 ± 1·0*	29·1 ↑	27·1 ± 1·0	7·4 ↑

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

complement component C_{5a} in ZAS. It is difficult to see how this reaction could be reduced by either cyclooxygenase or lipoxygenase inhibition, particularly the former as benoxaprofen is the only one of a group of NSAI which modifies the chemotactic response (Meacock et al 1979).

The more basic point of mechanism of action of NSAI would seem to continue as an open question. Clearly the actions of compounds such as benoxaprofen or BW755C (Higgs et al 1979) may relate in part to inhibition of cyclooxygenase or lipoxygenase, but there are still biological events which require explanation. Are the hydroxy-fatty acids chemotactic for monocytes, or are lipoxygenase and its products solely related to PMNs? Is cell movement a function of arachidonate metabolism or are there other biochemical events which are more relevant to emigration of PMN, monocytes or lymphocytes?

It has been suggested that a more useful concept in the description of a pharmacological agent, particularly in the anti-inflammatory area, is that of spectrum of activity (Dawson 1979b). The data reported here would support this concept as it is clear that there are other aspects of benoxaprofen activity the mechanism of which is unknown and it is not yet possible to assign a specific mode of action to this compound,

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Temporal response of prolyl hydroxylase in pre-existing granuloma to glucocorticoid administration*

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Administration of triamcinolone diacetate, a synthetic fluorinated glucocorticoid, results in a decrease of prolyl hydroxylase, the enzyme catalysing the hydroxylation of certain prolyl residues in collagen to form hydroxyproline, in liver, lung, heart, aorta and skin (Cutroneo et al 1975; Newman & Cutroneo 1978; Oikarinen 1977). However, reports from other laboratories have indicated that prolyl hydroxylase activity is not altered in both bone (Uitto & Mustakallio 1971) and in pre-existing granuloma tissue (Nakagawa et al 1971; Nakagawa & Tsurufuji 1972) following glucocorticoid treatment. We have determined the temporal response of prolyl hydroxylase to steroid treatment in pre-existing granuloma tissue, liver and skin tissues. Our data indicate that enzyme activity decreases in skin and granuloma tissues following multiple daily injections of drug. After a single injection of drug, only the liver enzyme activity was decreased.

Intact 90–110 g male albino Sprague-Dawley rats were supplied by Carsworth Farms, Rockland County, N.Y. All chemicals were analytical reagent grade. Commercial corticoid preparations were: Hydrocortisone acetate (Hydrocortone; Merck, Sharp and Dohme, West Point, Pa.); betamethasone maleate (Schering Corp., Bloomfield, N.J.). Powdered triamcinolone diacetate was supplied by Dr E. W. Cantrell of Lederle Laboratories, Pearl River, N.Y. Saline suspensions of the corticoids were prepared by adding the steroid to 0·9% (w/v) NaCl (saline) at a concentration of 5 mg ml^{-1} and mixing with a Potter-Elvehjem homogenizer fitted with a Teflon-coated pestle to a smooth suspension.

Enzyme activities in the 15 000 *g* supernatant from homogenates of liver, skin and granuloma tissues were measured by a modification (Cutroneo et al 1975) of the tritium release assay of Hutton et al (1966) using chick embryo substrate. Tritium release was linearly related to the time of incubation and was proportional to the amount of enzyme. Final radioactivity in the experimental samples was 4 to 5 times the radioactivity of the blank prepared by incubating the substrate without enzyme. All enzyme assays were carried out on at least two levels of 15 000 *g* supernatant to ensure linearity in relation to enzyme concentration. Enzyme activities can only be compared within a Table or figure since different substrates having different activities for the same enzyme preparation were used throughout these studies. Protein concentration in the 15 000 *g* supernatant was determined (Lowry et al 1951) using bovine serum albumin (Calbiochem, Los Angeles, Calif.) as standard.

Sub-dermal implants of polyvinyl sponges were used to induce granuloma tissue growth. The sponges (10 mm diam. 42 mm length, Scientific Products) were washed in running water for 48 h, sterilized for 15 min and dried. Before implantation they were rinsed in sterile saline. The animals were anaesthetized with ether. A trocar was inserted and guided to the dorsothoracic region and the sponge implanted. On the fifth day after sponge implantation, the animals were administered 50 mg kg^{-1} i.p. of the corticoid suspension (a dose of 20 mg kg^{-1} had not decreased prolyl hydroxylase activity in carregeenan-induced granuloma tissue, Nakagawa et al 1971, nor was any decrease observed in chick embryo tibiae of birds receiving a dose of approximately 100 mg kg^{-1} (Uitto & Mustakallio 1971). Controls were treated with saline. The animals were killed by cervical dislocation 12 h after a single injection or 24 h after the last of several injections. The granuloma tissue was removed from the sponge and weighed frozen in liquid nitrogen and pulverized. A 10% (w/v) homogenate in 0·25 M sucrose, 10^{-5} M ethelenediamine tetracetic acid, 10^{-5} M dithiotheritol, and 0·05 M Tris-HCl (pH 7·5) was prepared using a Polytron ST-10 system (Kinematica GmbH, Luzern, Switzerland). The homogenates were centrifuged for 15 min at 4 °C in an International B-60 centrifuge at 15 000 *g* and the supernatant used as the enzyme source. All animals were killed at 9 a.m. to avoid diurnal variation (Cutroneo & Scott 1973). A 2·5 cm square area of the ventral abdominal skin was

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