Br. J. Pharmac. (1985), **86**, 417–426

Oxidative transformations of arachidonic acid in human dispersed lung cells: disparity between the utilization of endogenous and exogenous substrate

J. Harvey*, S.T. Holgate, B.J. Peters*, C. Robinson[1] & J.R. Walker*

Medicine I, Centre Block, Southampton General Hospital, Southampton SO9 4XY, Hampshire and Lilly Research Centre Ltd*, Eli Lilly & Co., Erl Wood Manor, Windlesham, Surrey GU20 6PH

1 Eicosanoid release from human dispersed lung cells (HDLC) containing ca 5% mast cells was studied before and after cell activation with ionophore A23187 or anti-IgE.

2 Basal release of eicosanoids synthesized from endogenous arachidonate was measured by radioimmunoassay. In descending order of abundance the products were: 5-hydroxyeicosatetraenoic acid (5-HETE) > thromboxane B_2 (TXB_2) > prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) ≈ immunoreactive (i)-PGE_2 > PGD_2 > 6-keto-$PGF_{1\alpha}$ ≈ i-LTC_4.

3 Stimulation of HDLC with ionophore A23187 or, after passive sensitization, with anti-IgE resulted in 2–10 fold increases in the generation of individual eicosanoids. In terms of net generation the most abundant products were PGD_2 and TXB_2 with either stimulus. Activation with A23187 caused net release of i-LTC_4 and 5-HETE, but these products were not measured after immunological activation.

4 A more complete profile of lipoxygenase products released from HDLC dispersed from one lung was obtained after separation by high performance liquid chromatography combined with ultra violet spectroscopy and bioassay. The major products released from the cells from this lung with ionophore stimulation were 13-hydroxylinoleic acid > LTB_4 > 5-HETE > 12-HETE > LTC_4 > 15-HETE > 11-HETE ≈ 9-HETE.

5 When the utilization of exogenous [^{14}C]-arachidonic acid for prostanoid biosynthesis was compared to that of endogenous unlabelled arachidonate the formation of TXB_2 was consistently underestimated. These results imply compartmentalization of arachidonic acid utilization in Ca^{2+}-activated HDLC.

6 In unstimulated cells the proportional formation of PGD_2 was overestimated when exogenous arachidonic acid was substrate. After activation with A23187 the proportions of PGD_2 were similar with both substrate sources.

7 The large proportions of PGD_2 and TXB_2 generated by HDLC further supports the view that these eicosanoids may be important inflammatory mediators in lung tissue.

Introduction

Immunological activation of human pulmonary mast cells leads to the generation of a large number of inflammatory mediators, among which are products of the oxidative metabolism of arachidonic acid. The major cyclo-oxygenase product released from purified human lung mast cells is prostaglandin D_2 (PGD_2; Lewis *et al.*, 1982; Schleimer *et al.*, 1983; Holgate *et al.*, 1984). This prostanoid may be of particular importance in asthma because, when given by inhalation, it is a potent bronchoconstrictor in man and asthmatic subjects show enhanced reactivity to its effects (Hardy *et al.*, 1984). In addition to prostaglandin D_2, activated human lung mast cells also release sulphidopeptide leukotrienes (MacGlashan *et al.*, 1982; Peters *et al.*, 1984), although some workers (Paterson *et al.*, 1976) have claimed that a secondary cell type is essential for optimum production. Despite the continuing controversy regarding the mast cell origin of sulphidopeptide leukotrienes, an examination of their pharmacological actions suggests that they could also be important inflammatory mediators in bronchial

[1] Correspondence.

asthma (for reviews see Dahlén, 1983; Lewis & Austen, 1984; Robinson & Holgate, 1985).

Enzymatic digestion of human lung tissue disperses a population of cells essentially devoid of elements of bronchial and vascular smooth muscle. The majority of these inflammatory cells comprise macrophages (7–32%) with smaller amounts of lymphocytes (2–5%), mast cells (2–9%) and granulocytes (0.1–14.3%). The remainder of the cells comprise mainly pneumocytes. When these preparations are stimulated either immunologically or with ionophore A23187, large quantities of other eicosanoids are generated and released. These include thromboxane B_2 (TXB_2) and smaller quantities of prostaglandins E_2, $F_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (Holgate *et al.*, 1984). Purification of dispersed lung cell preparations suggests that the major source of released thromboxane are cells of the monocyte-macrophage series (Holgate *et al.*, 1984). Until recently, there have been no published studies which have attempted a detailed, systematic and quantitative analysis of both lipoxygenase and cyclo-oxygenase products from human lung cells. There are a number of reasons which may account for this. Firstly, such studies require a regular supply of human tissue and the problems associated with the measurement of a large number of related eicosanoids are great. Secondly, in view of the small amounts of some mediators released, radioimmunoassay is often the sole means of quantitative analysis applicable. One study has attempted to circumvent this problem by prelabelling cells with tritiated arachidonic acid (Peters *et al.*, 1984). However, prelabelling experiments may be unsatisfactory as they require prolonged incubation with large quantities (50–100 μCi) of tritium labelled arachidonic acid, during which time appreciable tritium exchange may occur. Isotope conversion may nevertheless be a useful technique to evaluate the pharmacology of lipoxygenase and cyclo-oxygenase inhibitors in human cell systems, and we describe investigations to study the utilization of [^{14}C]-arachidonic acid when given as a pulse with cell activation compared to the fate of endogenous arachidonate. Some of this work has been presented in abstract form at the Ninth International Congress of IUPHAR.

Methods

Preparation of mast cell-enriched dispersed lung cells

Fresh human lung tissue (usually 50–100g) obtained at thoracotomy was chopped finely and subjected to three sequential 30 min proteolytic digestions with pronase (2 mg ml^{-1}) and chymopapain (0.5 mg ml^{-1}). All digestions were performed at 37°C in modified Tyrode solution (composition, mM: NaCl 137, glucose 5.5, NaH_2PO_4 0.4, KCl 2.7, $MgCl_2$ 0.5, $CaCl_2$ 2.5) buffered to pH 7.4 with 20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) and containing 0.03% human serum albumin (HSA). Mast cell-enriched human dispersed lung cells (HDLC) obtained at the end of each digestion were separated from residual tissue fragments by sieving through 60 μm gauze filters and combined in the ratio 0.2:1:1 (v:v:v). Only a small proportion of cells from the first digestion was used in order to reduce erythrocyte contamination which is less marked in subsequent digests. HDLC were then washed twice in modified Tyrode solution buffered with 10 mM HEPES. The pooled cells were gently resuspended in Tyrode solution containing 0.1% gelatin and 0.02 mg ml^{-1} deoxyribonuclease (TGD) to reduce cell aggregation, and incubated at room temperature for 60 min. Cells for immunological release studies were then centrifuged at 1000 *g* for 15 min and passively sensitized with human IgE for 15 min at 37°C by resuspension in 1 ml of atopic serum. After extensive washing, all HDLC preparations were resuspended in albumin-free TGD and aliquots placed in Eppendorf tubes to give a final concentration of approximately 10^7 nucleated cells ml^{-1}. Mast-cells were counted after metachromatic staining of wet preparations with Kimura's stain.

Cell activation

Cells, prewarmed to 37°C for 5 min were challenged in a final volume of 1 ml with either sheep IgG ε-chain specific anti-human IgE (1:10 dilution); 20 μl ionophore A23187 (final concentration 2.5 μM) in aqueous dimethylsulphoxide vehicle, or dimethylsulphoxide solution or Tyrode buffer alone. Investigations showed that dimethylsulphoxide did not affect the release or assay of eicosanoids at the concentrations employed (0.2%). To study the fate of exogenous arachidonic acid some HDLC preparations were challenged simultaneously with 1 μCi of [1-^{14}C]- arachidonic acid dissolved in TGD. Reactions were allowed to proceed for 20 min and then terminated by centrifugation (Beckman Microfuge) at 10,000 *g* for 30 s. Our previous studies have demonstrated that this is the optimal time for the generation of prostaglandins (Holgate *et al.*, 1984). Supernatant fractions for radioimmunoassay (RIA) were frozen to − 20°C until assayed, while those for thin layer radiochromatography (radio-t.l.c.) were extracted and analysed as described below.

Extraction and thin layer radiochromatography

Incubation supernatants were extracted at pH 8.0 on C_{18} Sep Pak cartridges (Waters Associates, Northwich, Cheshire) which had been preconditioned with

20 ml methanol and 20 ml distilled water. After sample loading, the cartridges were washed with 5 ml ethanol :water (1:9 v:v) and eicosanoids eluted with 10 ml methanol. The percentage recovery of tritiated eicosanoids through this procedure was as follows: prostaglandin D_2 (PGD_2) 80.1 ± 2.5, PGE_2 62.3 ± 3.8, $PGF_{2\alpha}$ 71.8 ± 2.3, TXB_2 88.1 ± 2.2, leukotriene B_4 (LTB_4) 65.2 ± 5.4, LTD_4 71.8 ± 3.2, 5-hydroxy-eicosatetraenoic acid (5-HETE) 83.1 ± 3.8, 15-HETE 74.5 ± 3.3 (n = 5–17 experiments). The extracts were reduced to a volume of 50 µl under nitrogen and applied to separate tracks of a t.l.c. plate (Merck Silica gel 60, 20–20 cm coating thickness 0.025 cm). Plates were developed to 15 cm in toluene: 1,4-dioxan:acetic acid (65:34:1.5 v:v:v) at 4°C, as described by Harvey & Osborne (1983). Non-destructive quantitative analysis of the radioactive products was performed using a Berthold LB284 linear analyser interfaced to an Apple IIe microcomputer. Radioactive peaks were identified by reference to authentic radiolabelled standards applied to separate tracks of each plate. In view of the similar recoveries for the arachidonic acid metabolites measured, no corrections have been applied for losses incurred during extraction of these products as data are subsequently presented in terms of relative proportions.

High performance liquid chromatography (h.p.l.c.)

H.p.l.c. separation of eicosanoids obtained from HDLC incubation media was performed using a Spectra-Physics SP8700 ternary pumping system and variable wavelength ultravioilet (u.v.) detector. Lipoxygenase products were separated on a 12.5 × 0.46 cm internal diameter column packed with Techsphere 5 ODS (HPLC Technology Ltd, Macclesfield, Cheshire) using a mobile phase comprising methanol: water:acetic acid, 65:35:0.06 (v:v:v), adjusted to pH 5.3 with ammonia (sp.gr. 0.88 g ml^{-1}) (Osborne *et al.*, 1983). The flow rate was 1 ml min^{-1}. The column was calibrated for retention times using authentic unlabelled standards of lipoxygenase products and additional confirmation of product identity obtained by u.v. scanning in the stopped-flow mode. Sulphidopeptide leukotrienes and leukotriene B_4 were quantified using comparison with peak areas of standards of known concentration by h.p.l.c. and bioassay as described elsewhere (Baker *et al.*, 1981, Boot *et al.*, 1985).

Radioimmunoassay (RIA)

Radioimmunoassay of prostaglandins D_2, E_2, $F_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 were performed as described previously (Holgate *et al.*, 1984). Radioimmunoassay kits for leukotriene C_4 were purchased from New England Nuclear (Southampton, Hampshire). Cross-reactivity of the LTC_4 antibody was as follows: (5S,6R)-LTC_4 and (5R,6R)-LTC_4 100%; 11-*trans*-LTD_4 60.5%; LTD_4 55.3%; LTD_4 sulphone 10.1%; LTC_4 sulphone 9.5%, LTE_4 8.6% and LTE_4 sulphone 2.3%; 5-HETE 0.07%; LTB_4 0.005%; TXB_2 0.0002%; PGD_2 < 0.0002%. 5-HETE was assayed with RIA kit (Metachem Diagnostics, Piddington, Northans). Cross-reaction with heterologous ligands was: 5-HETE-δ-lactone 50%; LTB_4, C_4, D_4, E_4 3.5%; 15- and 12-HETEs < 0.1–0.6%; cyclo-oxygenase products < 0.1%. As the PGE_2 antibody employed showed cross-reaction with PGE_1, and the LTC_4 antibody cross-reacts appreciably with heterologous leukotriene ligands, we state levels for each compound as immunoreactive (i-) PGE_2 or LTC_4.

Statistical analysis

Data for eicosanoid generation and release from endogenous substrate are normalized per million mast cells and corrected for spontaneous release unless stated otherwise. Results from exogenous ^{14}C substrate studies are presented for each compound as the percentage of the total radioactivity present in individual tracks of the radiochromatogram. Significance of differences were evaluated using Student's two-tailed t test for paired data.

Materials

The following were purchased from Sigma Chemicals (Poole, Dorset): Papaya latex chymopapain, pronase type XIV, deoxyribonuclease fraction V from bovine pancreas, dextran, HEPES, ionophore A23187, dimethylsulphoxide and gelatin type I from swine skin. Human serum albumin was obtained from the Blood Products Laboratory (Elstree, Hertfordshire). Activated charcoal and all other standard reagents were purchased from BDH Chemicals (Poole, Dorset). Solvents for h.p.l.c. were purchased from Fisons PLC (Loughborough). Unlabelled cyclo-oxygenase products were generous gifts of the Upjohn Co. (Kalamazoo, MI, U.S.A.) and unlabelled leukotrienes were generously provided by Dr J. Rokach, Merck-Frosst Laboratories, Pointe-Claire, Canada. The following isotopes were purchased from Amersham International PLC (Amersham, Bucks): [5,6,8,9,12,14,15-(n)-^3H]-PGD_2 (100 Ci $mmol^{-1}$), [5,6,8,9,11,12,14,15-(n)-^3H]-TXB_2 (155 Ci $mmol^{-1}$), [1-^{14}C]-arachidonic acid (57–60 mCi $mmol^{-1}$), 6-keto-[5,8,9,11,12,14,15-(n)-^3H]-$PGF_{1\alpha}$ (120 Ci $mmol^{-1}$), [5,6,8,9,11,12,14,15-(n)-^{3}n]-$PGF_{2\alpha}$ and PGE_2 (160–180 Ci $mmol^{-1}$). 5-D-[5,6,8,9,11,12,14,15-(n)-^3H]- hydroxy-6,8,11,14-eicosatetraenoic acid (80 Ci $mmol^{-1}$), 15-L-[5,6,8,9,11,12,14,15-(n)-^3H]-hydroxy-5,8,11,13-eicosatetraenoic acid (62 Ci $mmol^{-1}$), [14,15-(n)-^3H]-LTC_4

and LTD_4 (40–50 Ci mmol^{-1}) were purchased from New England Nuclear (Southampton, Hants).

Results

Formation of eicosanoids from endogenous substrate

In the absence of challenge, HDLC containing 5.0 ± 0.7% (mean ± s.e.mean, $n = 19$ lungs) mast cells and released the following eicosanoids in descending order of abundance: 5-hydroxyeicosatetraenoic acid (5-HETE) > TXB_2 > $PGF_{2\alpha}$ ≈ i-PGE_2 > PGD_2 > 6-keto-$PGF_{1\alpha}$ ≈ i-LTC_4 (Tabl 1). When the cells were activated with the calcium ionophore A23187, there were 2–10 fold increases in the generation of individual eicosanoids. When expressed in terms of net generation, i.e. corrected for spontaneous release, the two predominant products were PGD_2 and TXB_2 which rose 9 and 5 fold respectively with cell stimulation (Table 1, $P < 0.05$–0.01). In cells from some lungs 6-keto-$PGF_{1\alpha}$ was present in substantial amounts, rising ten fold after A23187 challenge. However, the extent of this response was variable as reflected in the large standard error of the mean (Table 1). Stimulation of HDLC with A23187 also resulted in the net generation of the 5-lipoxygenase metabolites i-LTC_4 and 5-HETE, although in the latter case the two fold rise was not statistically significant (Table 1).

When passively sensitized HDLC were activated immunologically with a 1:10 dilution of sheep ε-chain specific anti-human IgE the most abundant cyclooxygenase product was TXB_2 which rose six fold with immunological challenge. Under these conditions HDLC generated less PGD_2 than with A23187, although this may be related to the relatively poorer net release of histamine: 9.8 ± 4.5% ($n = 7$) compared to 39.7 ± 9.8% ($n = 7$) with ionophore. Immunological activation also resulted in the generation of less 6-keto-$PGF_{1\alpha}$, but the net release of i-PGE_2 and $PGF_{2\alpha}$ was similar to the release with A23187 (Table 1). The lipoxygenase products i-LTC_4 and 5-HETE were not measured in these experiments.

Table 1 The release of eicosanoids from endogenous arachidonate in mast cell-enriched human dispersed lung cells (HDLC) activated with ε-chain specific anti-human IgE (1:10) or with ionophore A23187 (2.5 μM)

	Release (ng per 10^6 mast cells)		
Eicosanoid	*Spontaneous*	*Net ionophore*	*Net immunological*
PGD_2	3.31 ± 0.39	25.19 ± 6.93[b]	11.10 ± 4.00[a]
i-PGE_2	4.8 ± 0.8	3.49 ± 1.25	3.0 ± 1.2
$PGF_{2\alpha}$	5.14 ± 1.19	2.24 ± 0.72	2.70 ± 1.51
TXB_2	6.55 ± 1.28	24.49 ± 7.65[b]	33.80 ± 9.90[b]
6-keto-$PGF_{1\alpha}$	1.7 ± 0.8	16.70 ± 8.20	0.8 ± 0.8
i-LTC_4	1.65 ± 0.69	2.72 ± 0.29[c]	NM
5-HETE	7.74 ± 1.57	8.57 ± 3.81	NM

NM = not measured. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ with respect to spontaneous release. In this subsequent Tables: PGD_2 = prostaglandin D_2; i-PGE_2 = immunoreactive-prostaglandin E_2; TXB_2 = thromboxane B_2; i-LTC_4 = immunoreactive-leukotriene C_4; 5-HETE = 5-hydroxyeicosatetraenoic acid.

Formation of eicosanoids from exogenous arachidonic acid

Addition of 1 μCi of [1-^{14}C]-arachidonic acid to suspensions of HDLC resulted in the cellular incorporation of radiolabel as revealed by extracting whole cell suspensions directly into ethyl acetate at pH 3.0. Phospholipids and neutral lipids accounted for 12–56% of the total radioactivity, while 25–30% of label was associated with free arachidonic acid (range of two observations). However, in routine experiments the incubation medium was extracted at pH 8.0 into methanol using C_{18} Sep Paks, as our preliminary studies showed this to be a better single step extraction system for mixtures of prostaglandins and leukotrienes. Analysis of the radioactivity present in the incubation medium 20 min after addition of arachidonic acid showed that 25.4 ± 6.0% ($n = 6$) of the label added was present in the incubation medium. Unchanged arachidonic acid comprised the majority of this material (74.2 ± 4.1%, $n = 15$, Table 2), the rest being oxidative metabolites. Thus, of the label added only 6.6% had been utilized and released into the extracellular medium as eicosanoids. According to this

Table 2 Conversion of exogenous [^{14}C]-arachidonic acid to labelled products by mast cell-enriched human dispersed lung cells

	Percentage of total radioactivity		
Eicosanoid	*Unchallenged*	*A23187**	*Anti-IgE***
PGD_2	3.5 ± 0.9	6.9 ± 1.7	3.8 ± 1.3
PGE_2	0.9 ± 0.3	0.4 ± 0.1	1.5 ± 0.9
$PGF_{2\alpha}$	0.9 ± 0.3	1.0 ± 0.2	1.5 ± 0.9
TXB_2	1.1 ± 0.3	1.9 ± 0.4	1.5 ± 0.7
HHT	1.4 ± 0.3	1.8 ± 0.4	0.9 ± 0.3
Polar peak	7.3 ± 1.7	8.8 ± 1.6	12.1 ± 1.3
5-HETE	3.5 ± 0.8	4.6 ± 1.0	3.9 ± 0.5
mono-HETEs	1.9 ± 0.5	3.2 ± 0.6	1.0 ± 0.3
5,12-diHETEs	1.3 ± 0.4	1.9 ± 0.5	1.6 ± 0.6
Arachidonic acid	74.2 ± 4.1	69.8 ± 5.3	71.3 ± 4.4

Data are mean ± s.e.mean from 15 separate lungs except for anti-Ige activation in which $n = 5$. *2.5 μM, **1:10 final dilution.

method PGD_2 was the most abundant cyclo-oxygenase product comprising 3.5 ± 0.9% (n = 15) of the total supernatant radioactivity. Smaller amounts of ^{14}C were associated with HHT, PGE_2, $PGF_{2\alpha}$ and TXB_2 (Table 2). There was no detectable formation of 6-keto-$PGF_{1\alpha}$ in these experiments. Of the lipoxygenase products formed by HDLC less than 4% of each 5-HETE, mono-HETEs and 5,12-diHETEs was measured. A large peak was consistently observed at the origin of the chromatogram, and thus referred to as the polar peak. When extractions were performed at pH 8.0 this peak accounted for 7.3 ± 1.7% of the total superantant radioactivity (n = 15; Table 2). However, when supernatants were extracted at pH 3.0 into ethyl acetate the size of the polar peak was reduced to 0.7% (n = 7) of the total ^{14}C present in the supernatant. In contrast, prostaglandins and hydroxyeicosatetraenoic acids were efficiently extracted with 62–88% recovery (n = 15) under these conditions.

When HDLC were challenged simultaneously with A23187 and [1-^{14}C]-arachidonic acid there were small increases in the proportions of most products at the expense of arachidonic acid and PGE_2. The largest rise was seen with PGD_2 which composed 6.9 ± 1.7% of supernatant radioactivity (Table 2). Immunological activation of HDLC with ε-chain specific anti-human IgE resulted in very similar increases in the proportions of all metabolites with the exclusion of PGD_2, HHT and mono-HETEs (Table 2).

Confirmation of lipoxygenase products formed from exogenous substrate

In order to confirm the identity of the lipoxygenase products formed from [1-^{14}C]-arachidonic acid by HDLC, Sep Pak extracts of incubation media were analysed by h.p.l.c. and u.v. detection. Where possible u.v. absorbance spectra were obtained for peaks eluting with the retention times of know lipoxygenase products. Biological characterization and quantification of sulphidopeptide leukotrienes were performed using guinea-pig ileum smooth muscle preparations, while the biological activity of LTB_4 was confirmed using a guinea-pig neutrophil aggregation assay. The separation of eicosanoids obtained by h.p.l.c. is illustrated in Figure 1.

A large sample of lung tissue was needed to perform these investigations and this limited the number of experiments which could be performed. Table 3 shows the results from two separate studies performed on cells obtained from one 280 g specimen of lung tissue. These incubations were performed in a total volume of 2 ml with a final cell concentration of 45.6×10^6 nucleated cells per ml. Mast cell content was 5.6%, the remainder being cells of the monocyte-macrophage series with smaller numbers of pneumocytes, eosinophils and neutrophils. In the absence of challenge HDLC released small quantities of LTB_4 and mono-hydroxyeicosatetraenoic acids which were associated

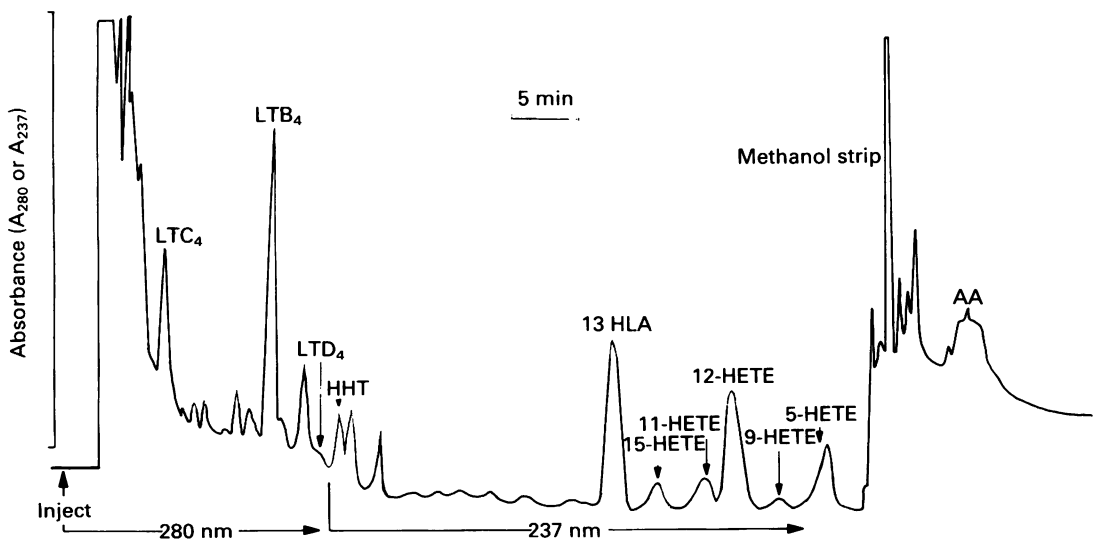

Figure 1 Separation of lipoxygenase products released by ionophore A23187 (2.5 μM) from human dispersed lung cells. Mobile phase composition: methanol/water/acetic acid, 65/35/0.06, adjusted to pH 5.3. Analysis was performed on a Techsphere 5 ODS column (12.5 cm × 0.46 cm) at a flow rate of 1 ml $min.^{-1}$ Detector wavelength was changed from 280 nm to 237 nm at the point indicated. LTB_4, C_4 and D_4 = leukotriene B_4, C_4 and D_4, respectively; 13 HLA = 13-hydroxylinoleic acid; 5-,9-,11-, 12- and 15-HETE = 5-,9-,11-,12-and 15-hydroxyeicosatetraenoic acid, respectively; AA = arachidonic acid.

Table 3 Formation of eicosanoids and utilization of [^{14}C]-arachidonic acid by human dispersed lung cells in the absence and presence of A23187 (2.5 μM) when products were separated by h.p.l.c.

Eicosanoid	*Unchallenged* (ng per 10^6 MC)	*% total SN* radioactivity	*Specific activity* (nCi nmol^{-1})	*+ Ionophore* (ng per 10^6 MC)	*% total SN radioactivity*	*Specific activity* (nCi nmol^{-1})
LTB_4	3.3	0.8	4.7	41.1	6.0	4.0
LTC_4	ND	0.7	—	26.8	3.5	6.4
LTD_4	ND	0.6	—	1.0	1.3	49.5
6-trans-LTB_4	1.2	0.2	3.5	8.2	1.0	3.1
5-HETE	5.3	0.4	1.6	31.9	3.8	3.2
9-HETE	1.8	0.1	1.4	4.5	0.5	3.2
11-HETE	9.2	0.4	1.8	4.7	0.7	2.0
12-HETE	9.0	0.9	2.0	28.2	7.1	7.5
15-HETE	3.7	0.5	2.8	9.4	0.9	2.6
13-HLA	20.9	0	0	52.4	0	0
HHT	7.4	2.9	6.5	20.4	3.6	4.0
Prostaglandins	NM	17.7	—	NM	23.6	—

Results are means of two separate experiments on cells obtained from one 280 g specimen of lungs. Product separation was by h.p.l.c. ND = not detected; NM = not measured; SN = supernatant incubation medium; MC = mast cell.

with only small amounts of ^{14}C (Table 3). In addition HDLC also spontaneously released HHT and 13-hydroxylinoleic acid (13-HLA). The latter compound was not associated with any ^{14}C in the h.p.l.c. column effluent. The spontaneous release of individual prostanoids was not measured in these experiments as the mobile phase employed does not differentiate between prostaglandin classes. However, the prostaglandins comprised 17.7% of the total supernatant radioactivity (Table 3). Stimulation of the HDLC with A23187 resulted in the net release of LTB_4, LTD_4, 9-HETE, 12-HETE, 15-HETE, HHT and 13-HLA which, with the exception of 13-HLA, was associated with an increase in the percentage of the total radioactivity for each compound (Table 3). The proportion of radioactivity associated with the prostaglandin fraction was increased from 17.7% to 23.6% with ionophore stimulation. Examination of the specific activity of the radiolabelled products revealed that ionophore stimulation resulted in small increases in the activity of 5-HETE and 9-HETE. The specific activities of 11-HETE and LTB_4 were unchanged by ionophore stimulation (Table 3), but it was not possible to measure changes in the specific activity of the sulphidopeptide leukotrienes as unchallenged release was undetectable by h.p.l.c. or bioassay. In contrast the specific activity of HHT was decreased slightly after challenge with A23187.

Comparative utilization of endogenous and exogenous arachidonic acid

In this series of experiments the utilization of endogenous or exogenous arachidonic acid for prostanoid biosynthesis was compared in paired samples from six separate lungs. The data presented here have not been corrected for mast cell numbers (4–11%) but total nucleated cell numbers were held at $0.8-1.0 \times 10^7$ cells per ml. With endogenous arachidonate as substrate, unchallenged cells released TXB_2 and $PGF_{2\alpha}$ as their major products (4.34 ± 0.71 and 4.30 ± 0.71 ng ml^{-1} supernatant respectively), with smaller quantities of PGD_2 (2.85 ± 0.68 ng ml^{-1}) and i-PGE (2.07 ± 0.70 ng ml^{-1}). Activation of these cells with 2.5 μM

Table 4 Comparison of the utilization of exogenous and endogenous arachidonic acid in human dispersed lung cells in the absence and presence of 2.5 μM A23187

	*Exogenous**		*Endogenous***	
Prostanoid	*Unchallenged*	*Ionophore*	*Unchallenged*	*Ionophore*
PGD_2	63.0 ± 5.4	63.4 ± 7.0	19.7 ± 2.5	43.5 ± 7.9†
PGE_2	7.6 ± 5.0	5.62 ± 2.4	17.9 ± 1.1	6.4 ± 1.0†
$PGF_{2\alpha}$	14.2 ± 3.4	12.6 ± 2.4	31.5 ± 1.7	6.7 ± 1.5†
TXB_2	15.1 ± 3.5	18.4 ± 3.8	31.0 ± 0.7	41.5 ± 6.2

Data are presented for each compound as the percentage of the total measured prostanoid formation. Results have not been corrected for mast cell content as this is not possible for exogenous substrate studies. However, mast cell purity was 4–11% and total cell numbers were held at $0.8-1.0 \times 10^7$ cells ml^{-1}.

†$P < 0.05-0.001$ with respect to appropriate unchallenged control, $n = 6$ lungs.

*Measurements made by radio t.l.c.

**Measurements made by RIA.

A23187 resulted in an increased release of TXB_2 to $33.45 \pm 8.75\,ng\,ml^{-1}$ ($P<0.01$) and PGD_2 to $31.29 \pm 4.67\,ng\,ml^{-1}$ ($P<0.01$). In addition there was a small rise in i-PGE generation to $4.86 \pm 1.05 ng\,ml^{-1}$ ($P<0.02$). The generation of $PGF_{2\alpha}$ was not significantly different from control.

Table 4 shows the results of these experiments when the utilization of endogenous arachidonate, measured by RIA, was compared to exogenous substrate utilization. There were no significant differences in the relative proportions of labelled PGD_2, PGE_2, $PGF_{2\alpha}$ and TXB_2 measured in unchallenged or ionophore stimulated HDLC when exogenous [^{14}C]-arachidonic acid was employed as substrate. The most abundant product was PGD_2 which represented 63% of the total radioactivity associated with prostanoids, whereas TXB_2 comprised only 15–18% of the radioactive cyclo-oxygenase products measured. In contrast, it can be seen from Table 4 that in the paired samples used to measure endogenous substrate utilization PGD_2 and TXB_2 represented $19.7 \pm 2.5\%$ and $31.0 \pm 0.7\%$ of the prostanoids in unchallenged cells. Both of these values are significantly different ($P<0.001$) from the corresponding measurements made for exogenous substrate. A similar discrepancy is seen with $PGF_{2\alpha}$ where there is relatively greater synthesis from endogenous rather than exogenous substrate ($P<0.01$). Thus in the case of unstimulated TXB_2 and $PGF_{2\alpha}$ release, exogenous [^{14}C]-arachidonic acid significantly underestimates the formation of these substances, but for PGD_2 the reverse is true. However, when HDLC were subjected to activation with A23187 the only discrepancy between the two techniques was the proportion of TXB_2 present. With exogenous arachidonic acid TXB_2 represented $18.4 \pm 3.8\%$ of the cyclo-oxygenase products after ionophore activation (Table 4), but from endogenous arachidonic acid the proportion measured by RIA was greater ($41.5 \pm 6.2\%$, $P<0.01$). This difference is unlikely to be due to cross-reaction of the TXB_2 antibody with other eicosanoids as the antibody employed has a high specificity (Holgate *et al.*, 1984). Thus, these experiments demonstrate that even with cell activation exogenous substrate underestimates the total generation of TXB_2.

Discussion

In this study we have shown that mast cell-enriched human dispersed lung cells have the capacity to synthesize and release a wide array of cyclo-oxygenase and lipoxygenase products from endogenous or exogenous arachidonic acid. The most abundant cyclo-oxygenase products generated from endogenous arachidonate either by A23187 or anti-IgE were PGD_2 and TXB_2. Together these accounted for 69–87% of the total measured cyclo-oxygenase product output after cell activation, results which are in good agreement with our previous studies (Holgate *et al.*, 1984). In contrast, immunological or ionophore stimulation of human lung parenchymal fragments leads to the release of large amounts of PGI_2 (Schulman *et al.*, 1981). This probably originates from vascular endothelial or alveolar epithelial cells (Moncada *et al.*, 1977; Taylor *et al.*, 1979) which are absent in the dispersed cell system.

Preparations of HDLC activated with the calcium ionophore A23187 also released lipoxygenase products, as determined by RIA of 5-HETE and i-LTC_4. Additional confirmation of lipoxygenase product release was obtained by h.p.l.c. combined with u.v. absorbance detection and bioassay. Our findings differ from those of a previous study (Dahlén *et al.*, 1983) in which leukotriene formation was investigated in lung fragments from two subjects with birch pollen-sensitive asthma. In these experiments a large amount of 15-HETE production was noted, but in our own experiments it was only a minor product. One explanation for this discrepancy is that like PGI_2, the cells responsible for its biosynthesis are not present in HDLC in large numbers. The 15-HETE synthesized in HDLC probably originates from neutrophil and eosinophil leukocytes which comprise 0.3–13% and 0.1–1.3% of the total number of nucleated cells, assessed by staining with May-Grunwald-Giemsa. Experiments performed on human lung fragments have also suggested that human lung tissue releases a mixture of LTC_4, D_4 and E_4 (Lewis *et al.*, 1980). However, we found only small quantities of LTD_4 in one lung when lipoxygenase products were separated by h.p.l.c. and the effluent subjected to bioassay. Since the enzymes which mediate the further metabolism of LTC_4 are thought to be membrane bound (Morris *et al.*, 1982) it is possible that their activity is reduced after exposure to proteolytic enzymes.

The ability of human lung to synthesize and release TXA_2 is controversial. Three groups have claimed that this eicosanoid is not generated in signficiant amounts from either chopped human lung, perfused sections of parenchyma or pulmonary microsomes (Piper & Walker 1973., Al-Ubaidi & Bakhle 1980., Sun *et al.*, 1977). However, its release from human lung microsomes, purified human lung mast cells and cultured pulmonary fibroblasts has been found by other workers (Ally *et al.*, 1982; Schleimer *et al.*, 1983; Hopkins *et al.*, 1978), and in addition we have recently provided evidence (Holgate *et al.*, 1984) that important sources of TXB_2 are cells of the monocyte-macrophage series. In HDLC obtained from six lungs we consistently found that despite the release of large amounts of TXB_2 (measured by RIA), paired samples from the same lung incorporated only small amounts of exogenous [^{14}C]-arachidonic acid into this product.

It is unlikely that this discrepancy is due to loss of TXB_2 during extraction as [^{3}H]-TXB_2 standards were recovered in high yield. The failure of some studies (Piper & Walker, 1973; Al-Ubaidi & Bakhle, 1980) to detect thromboxane formation in intact human lung may be related to the poor processing of exogenous substrate by thromboxane synthetase. The contradictory findings obtained with microsomal preparations may possibly result from technical difficulties associated with the rapid decomposition of the PGH_2 substrate employed in such studies. The simplest interpretation of our data is that there is a differential handling of endogenous and exogenous substrate in both unchallenged and ionophore activated HDLC. Thus supply of exogenous arachidonic acid results in its conversion only by those enzymes of eicosanoid biosynthesis which are readily accessible or 'preactivated'. A number of precedents for this hypothesis exist in other tissues. For example, anatomical compartmentalization has been observed in human lung mast cells and macrophages in which exogenous arachidonic acid is preferentially taken up into cytoplasmic lipid bodies (Dvorak *et al.*, 1983). However, the relevance of these storage sites to arachidonic acid metabolism is not known, but they may represent a mechanism for removal of free arachidonic acid to sites which are not immediately coupled for oxidative metabolism to some or all eicosanoids. A similar uptake of arachidonic acid into anatomically or biochemically distinct storage sites that are uncoupled from prostaglandin biosynthesis occurs in rabbit hydronephrotic kidneys or guinea-pig lung, where there is only poor oxidative utilization of labelled arachidonic acid despite substantial eicosanoid release (Schwartzman *et al.*, 1981; Jose & Seale, 1979). In the case of hydronephrotic kidney, the prolonged depletion of arachidonic acid stores results in the eventual utilization of lipid-incorporated [^{14}C]-arachidonate, presumably as a result of inter-pool transfer (Schwartzman *et al.*, 1981). Biochemical compartmentalization may also exist for the cleavage of arachidonic acid from phospholipid pools. Thus mouse macrophages (Hsueh *et al.*, 1981) and guinea-pig lung (Robinson & Hoult, 1980) have been claimed to have functionally distinct pools of phospholipases involved in arachidonic acid release.

In all the radio-t.l.c. experiments we observed a polar peak which remained at the origin of the t.l.c. plate. At present we do not know the identity of this compound as we have been unable to achieve sufficient recovery from t.l.c. plates for further analysis. However, indirect evidence suggests that a major proportion of the polar material(s) comprising this peak are sulphidopeptide leukotrienes. In support of this, activated HDLC generate LTC_4 when measured by RIA or h.p.l.c./bioassay. Secondly, the incorporation of ^{14}C into materials, characterized as LTC_4 and LTD_4 by h.p.l.c. retention time, u.v. spectrum and biological activity, was a mean 4.8% of the supernatant radioactivity which compares favourably with the polar peak on radio-t.l.c. which comprised 7.9% of the recovered radiolabel. Thirdly, in six lungs the size of the polar peak is reduced by approximately 50% after preincubation of the HDLC with 1 μM U-60,257 (Robinson & Holgate, 1984), a drug known to inhibit the formation of sulphidopeptide leukotrienes (Bach *et al.*, 1982; Dahlén *et al.*, 1983). Finally, the peak was reduced to 0.46% ($n = 7$) of the extracted radiolabel when supernatants were extracted into ethyl acetate at pH 3.0. Under these conditions poor recovery and decomposition of authentic leukotrienes has previously been noted (Morris *et al.*, 1979; Westcott *et al.*, 1984).

In summary, we have demonstrated that mast cell-enriched HDLC respond to IgE- or calcium-dependent activation with the net synthesis and release of a wide array of eicosanoids with potent biological actions. However, when arachidonic acid utilization is measured using exogenous substrate there is an underestimation of TXB_2 formation by these cells. The propensity of HDLC to generate PGD_2 and TXB_2 confirms our previous observations and lends further support to the concept (Robinson & Holgate, 1985) that both of these bronchoconstrictor eicosanoids could be important inflammatory mediators in bronchial asthma.

We thank the Medical Research Council and the Wessex Medical School Trust for financial support. We are indebted to Mrs M. Dowling and Mrs S. Foulkes for typing the manuscript.

References

ALLY, A.I., BOUCHER, R., KNOWLES, M.R. & ELING, T.E. (1982). Metabolism of prostaglandin endoperoxide by microsomes from human lung parenchyma and comparison with metabolites produced by pig, bovine, rat, mouse and guinea-pig. *Prostaglandins*, **24**, 575–584.

AL-UBAIDI, F. & BAKHLE, Y.S. (1980). Differences in biological activation of arachidonic acid in perfused lungs from guinea-pig, rat and man. *Eur. J. Pharmac.*, **62**, 89–96.

BACH, M.K., BRASHLER, J.R., SMITH, H.W., FITZPATRICK, F.A., SUN, F.F. & McGUIRE, J.C. (1982). 6,9-deepoxy-6,9-(phenylimino)-$\Delta^{6,8}$-prostaglandin I, (U-60,257) a new inhibitor of leukotriene C and D synthesis: *in vitro* studies. *Prostaglandins*, **23**, 759–771.

BAKER, S.R., BOOT, J.R., JAMIESON, W.B., OSBORNE, D.J. & SWEATMAN, W.J.F. (1981). The comparative *in vitro* pharmacology of leukotriene D_4 and its isomers. *Biochem. biophys. Res. Commun.*, **103,** 1258–1264.

BOOT, J.R., KITCHEN, E.A., WALKER, J.R., HARVEY, J. & DAWSON, W. (1985). Directional movement of cells *in vivo* and *in vitro* under the influence of 5-lipoxygenase inhibitors. *Br. J. Dermatol.*, (in press).

DAHLÉN, S-E. (1983). Pulmonary effects of leukotrienes. *Acta physiol. scand.*, Suppl. **512,** 1–51.

DAHLÉN, S-E, HANSSON, G., HEDQVIST, P., BJORCK, T., GRANSTROM, E. & DAHLEN, B. (1983). Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C_4, D_4 and E_4. *Proc. natn. Acad. Sci. U.S.A.*, **80,** 1712–1716.

DVORAK, A.M., DVORAK, H.F., PETERS, S.P., SCHULMAN, E.S., MACGLASHAN, D.W. Jr., PYNE, K., HARVEY, S., GALLI, S.J. & LICHTENSTEIN, L.M. (1983). Lipid bodies: cytoplasmic organelles important to arachidonate metabolism in macrophages and mast cells. *J. Immunol.*, 131, **2965-2976.**

HARDY, C.C., ROBINSON, C., TATTERSFIELD, A.E. & HOLGATE, S.T. (1984). The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic men. *N. Engl. J. Med.*, **311,** 209–213.

HARVEY, J. & OSBORNE, D.J. (1983). A rapid method for detecting inhibitors of both cyclooxygenase and lipoxygenase metabolites of arachidonic acid. *J. Pharmac. Methods.*, **9,** 147–155.

HOLGATE, S.T., BURNS, G.B., ROBINSON, C. & CHURCH, M.K. (1984). Anaphylactic- and calcium-dependent generation of prostaglandin D_2 (PGD_2) thromboxane B_2, and other cyclooxygenase products of arachidonic acid by dispersed human lung cells and relationship to histamine release. *J. Immunol.*, **133,** 2138–2144.

HOPKINS, N.K., SUN, F.F. & GORMAN, R.R. (1978). Thromboxane A_2 biosynthesis in human lung fibroblasts WI-38. *Biochem. biophys. Res. Commun.*, **85,** 827–836.

HSEUH, W., DESAI, U., GONZALEZ-CRUSSI, F., LAMB, R. & CHU, A. (1981). Two phospholipase pools for prostaglandin synthesis in macrophages. *Nature*, **290,** 710–713.

JOSE, P.J. & SEALE, J.P. (1979). Incorporation and metabolism of [^{14}C]-arachidonic acid in guinea-pig lung. *Br. J. Pharmac.*, **67,** 519–526.

LEWIS, R.A. & AUSTEN, K.F. (1984). The biologically active leukotrines. Biosynthesis, metabolism, receptors, functions and pharmacology. *J. clin. Invest.*, **73,** 889–897.

LEWIS, R.A., AUSTEN, K.F., DRAZEN, J.M., CLARK, D.A., MARFAT, A. & COREY, E.J. (1980). Slow reacting substances of anaphylaxis: Identification of leukotrienes C-1 and D from human and rat sources. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 3710–3714.

LEWIS, R.A., SOTER, N.A., DIAMOND, P.T., AUSTEN, K.F., OATES, J.A. & ROBERTS, L.J. II. (1982). Prostaglandin D_2 generation after activation of rat and human mast cells with anti-IgE. *J. Immunol.*, **129,** 1627–1631.

MACGLASHAN, D.W. Jr., SCHLEIMER, R.P., PETERS, S.P., SCHULMAN, E.S., ADAMS, G.K. III., NEWBALL, H.H. & LICHTENSTEIN, L.M. (1982). Generation of leukotrienes by purified human lung mast cells. *J. clin. Invest.*, **70,** 747–751.

MONCADA, S., HERMAN, A., HIGGS, E.A. & VANE, J.R. (1977). Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the antithrombotic properties of vascular endothelium. *Thromb. Res.*, **11,** 323–324.

MORRIS, H.R., PIPER, P.J., TAYLOR, G.W. & TIPPINS, J.R. (1979). Comparative studies on immunologically and non-immunologically produced slow reacting substances from man, guinea-pig and rat. *Br. J. Pharmac.*, **67,** 179–184.

MORRIS, H.R., TAYLOR, G.W., JONES, C.M., PIPER, P.J., SAMHOUN, M.N. & TIPPINS, J.R. (1982). Slow reacting substance (leukotrienes). Enzymes involved in their biosynthesis. *Proc. natn. Acad. Sci. U.S.A.*, **79,** 4836–4842.

OSBORNE, D.J., PETERS, B.J. & MEADE, C.J. (1983). The separation of leukotrienes and hydroxyeicosatetraenoic acid metabolites of arachidonic acid by high performance liquid chromatrography (HPLC). *Prostaglandins*, **26,** 817–832.

PATERSON, N.A.M., WASSERMAN, S.I., SAID, J.W. & AUSTEN, K.F. (1976). Release of chemical mediators from partially purified human lung mast cells. *J. Immunol.*, **117,** 1356–1362.

PETERS, S.P., MACGLASHAM, D.W. Jr., SCHULMAN, E.S., SCHLEIMER, R.P., HAYES, E.C., ROKACH, J., ADKINSON, N.F. Jr. & LICHTENSTEIN, L.M. (1984). Arachidonic acid metaboism in purified human lung mast cells. *J. Immunol.*, **132,** 1972–1979.

PIPER, P.J. & WALKER, J.L. (1973). The release of spasmogenic substances from chopped human lung and its inhibition. *Br. J. Pharmac.*, **47,** 291–304.

ROBINSON, C. & HOLGATE, S.T. (1984). Modulation of arachidonic acid metabolism in dispersed human lung cells by the inhibitor of leukotriene formation U-60,257 (Piriprost). *Prostaglandins*, **28,** 648.

ROBINSON, C. & HOLGATE, S.T. (1985). Mast cell dependent inflammatory mediators and their putative role in bronchial asthma. *Clin. Sci.*, **68,** 103–112.

ROBINSON, C. & HOULT, J.R.S. (1980). Evidence for functionally distinct pools of phospholipase responsible for prostaglandin release from perfused guinea-pig lung. *Eur. J. Pharmac.*, **64,** 333–339.

SCHLEIMER, R.P., SCHULMAN, E.S., MACLASHAN, D.W. Jr., PETERS, S.P., HAYES, E.C., ADAMS, G.K. III., LICHTENSTEIN, L.M. & ADKINSON, N.F.. Jr. (1983). Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J. clin. Invest.*, **71,** 1830–1835.

SCHULMAN, E.S., NEWBALL, H.H., DEMERS, L.M., FITZPATRICK, F.A. & ADKINSON, N.F. Jr. (1981). Anaphylactic release of thromboxane A_2, prostaglandin D_2 and prostacyclin from human lung parenchyma. *Am. Rev. Resp. Dis.*, **124,** 402–406.

SCHWARTZMAN, M., LIBERMAN, E. & RAZ, A. (1981). Bradykinin and angiotensin II activation of arachidonic acid deacylation and prostaglandin E_2 formation in rabbit kidney. *J. biol. Chem.*, **256,** 2329–2333.

SUN, F.F., CHAPMAN, J.P. & MCGUIRE, J.C. (1977). Metabolism of prostaglandin endoperoxide in animal tissues. *Prostaglandins*, **14,** 1055–1074.

TAYLOR, L., POLGAR, P., MCATEER, J.S. & DOUGLAS,

W.M.J. (1979). Prostaglandin production by type II alveolar epithelial cells. *Biochim. biophys. Acta.*, **572,** 502–509.

WESTCOTT, J., CLAY, K.L. & MURPHY, R.C. (1984). Decomposition of leukotriene C_4. *J. Allergy clin. Immunol.*, **74,** 363–367.

(Revised March 9, 1985.
Revised May 20, 1985.
Accepted July 8, 1985.)