J. Physiol. (1979), **295**, *pp.* 445–455
With 4 text-figures
Printed in Great Britain

THE FATE OF EXOGENOUS ARACHIDONIC ACID IN GUINEA-PIG ISOLATED LUNG

BY FIRIAL AL-UBAIDI AND Y. S. BAKHLE

From the Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN

(*Received 19 January 1979*)

SUMMARY

1. The fate of [^{14}C]arachidonic acid perfused through the pulmonary circulation was studied in guinea-pig lungs perfused with Krebs solution.

2. Radioactivity in the lung effluent fell rapidly and by 10 min about 20% of the infused radioactivity had emerged.

3. Most (70%) of the effluent radioactivity was associated with products of cyclo-oxygenase activity, whereas in the lung tissue most of the retained radioactivity was present as phospholipid.

4. Radioactivity in phospholipid was distributed equally between three groups: phosphatidyl choline, phosphatidyl ethanolamine and the other phosphatides.

5. Addition of albumin to the Krebs solution perfusing the lung increased the proportion of effluent radioactivity to 50%, decreased the cyclo-oxygenase products but increased the label in phospholipid in lung.

6. Indomethacin, frusemide, bromcresol green and diethylcarbamazine all decreased biological activation of arachidonic acid.

7. Indomethacin, bromcresol green and diethylcarbamazine also decreased effluent radioactivity and cyclo-oxygenase products with minimal effects on the distribution of radioactivity in lung lipid.

8. It appears that the major metabolic pathway for exogenous arachidonic acid perfused through the pulmonary circulation was incorporation into phospholipid. Metabolism via cyclo-oxygenase only involved about 15% of the total substrate infused.

INTRODUCTION

Infusion of arachidonic acid through the pulmonary circulation of guinea-pig isolated lungs gives rise to a number of myotropic compounds in the lung effluent (Palmer, Piper & Vane, 1973), some of which have been identified as metabolites of arachidonic acid (Hamberg, Svensson, Hedqvist, Strandberg & Samuelsson, 1976). It is not clear whether these active metabolites are derived from the administered acid or from endogenous acid, possibly in phospholipid. Furthermore, some earlier experiments of ours (Y. S. Bakhle & D. I. Heinzelman, unpublished) had suggested that only a small proportion of infused arachidonic acid emerged from the lung in any form. In order to clarify the fate of exogenous arachidonic acid, we decided to investigate the distribution of radioactivity in lung and in effluent following infusion

0022-3751/79/5670-0971 $01.50

of [^{14}C]arachidonic acid in guinea-pig isolated lungs. A preliminary report of some of these findings has been made to the Physiological Society (Al-Ubaidi, Bakhle, Jose & Seale, 1978).

METHODS

Lungs from male guinea-pigs were prepared and perfused with Krebs solution (8 ml. min^{-1}) as described previously (Bakhle, Reynard & Vane, 1969). Bio-assay of lung effluent was carried out using rat stomach strip, rat colon, rabbit aorta and guinea-pig ileum (Vane, 1964). A mixture of antagonists (methysergide 200 ng. ml.$^{-1}$, mepyramine 200 n. ml.$^{-1}$, hyoscine 100 ng. ml.$^{-1}$, phenoxybenzamine 100 ng. ml.$^{-1}$ and propranolol 2 μg. ml.$^{-1}$; final concentrations, weight base. ml.$^{-1}$) was infused (0·1 ml. min^{-1}) over the assay tissues to increase specificity for arachidonic acid and its metabolites. Indomethacin (2 μg. ml.$^{-1}$) was infused over the assay tissues to prevent prostaglandin synthesis from exogenous and endogenous arachidonic acid (Eckenfels & Vane, 1972).

Arachidonic acid was stored in *n*-hexane under nitrogen at -20 °C. The residue, after evaporating the hexane with a stream of nitrogen, was taken up in 0·9% saline and converted to the sodium salt of arachidonic acid with sodium hydroxide. Radioactive arachidonic acid was similarly stored and dissolved. Mixtures of radioactive and non-radioactive arachidonic acid were made to provide solutions for infusion, yielding a final concentration of 4 μM-arachidonic acid in the pulmonary perfusate and a total of 0·2–2 μc per infusion. Infusions of arachidonic acid were made for 3 min at 0·1 ml. min^{-1} into the Krebs perfusate flow (8 ml. min^{-1}).

Where required, drugs, e.g. indomethacin, frusemide, bromcresol green and diethylcarbamazine were added by infusion (0·1–0·3 ml. min^{-1}) to the Krebs flow to yield the desired final concentrations. In some experiments, the Krebs solution was replaced by Krebs solution containing bovine serum albumin (1%, w/v). Such changes were made for at least 15 min before infusing arachidonic acid and continued for the whole of the experiment.

Lung effluent was collected in 1 min fractions during the infusion and for varying times after the infusion. Samples (1 ml. of these fractions) were mixed with 10 ml. scintillant A (see below) and radioactivity measured in a Packard liquid scintillation counter. In many experiments a single fraction was collected, a sample (1 ml.) taken to measure total radioactivity, and the remainder after extraction separated by thin layer chromatography into three major components, cyclo-oxygenase products, hydroxy fatty acids and arachidonic acid using the double development methods of Jose & Seale (1979). The term 'cyclo-oxygenase products' is meant to include thromboxanes, prostaglandins and prostacyclins, all derived from a common endoperoxide precursor, PGG_2, itself a product of cyclo-oxygenase action on arachidonic acid. The term also covers metabolic or chemical degradation products of thromboxanes, prostaglandins and prostacyclins, for instance, 6-oxo-$PGF_{1\alpha}$ derived from prostacyclin (PGI_2). The area on the chromatogram denoted as cyclo-oxygenase products, outlined in Fig. 2 by the dotted lines, was defined by using $PGF_{2\alpha}$, E_2 and D_2 as markers and including all the radioactivity from $PGF_{2\alpha}$ up to the ricinoleic acid marker. Previous experiments had showed that 6-oxo-$F_{1\alpha}$, 15-oxo and 15-oxo-13, 14-dihydro derivatives of prostaglandins and thromboxane B_2 all fell within this area using our solvent systems. One derivative of the endoperoxide precursor, 12-hydroxy heptadecatrienoic acid (HHT) would not be included in the cyclo-oxygenase products area. Ricinoleic acid was the marker for this hydroxy acid and that derived from arachidonic acid via lipoxygenase, HETE.

At the end of the perfusion, radioactivity in the lung was measured. The lungs were weighed, homogenized in 17 vol. cold (0 °C) chloroform:methanol (2:1, v/v) and filtered through glass wool. The homogenizer and filter were rinsed with a further 2 vol. chloroform:methanol. After adding 0·2 vol. 0·12 M-KCl to the filtrate, the organic phase was removed. To recover (any) prostaglandins, the aqueous phase was acidified to pH 3·5 with hydrochloric acid and extracted with chloroform. This organic phase was added to the original and evaporated to dryness. To remove any remaining traces of water (which would interfere with subsequent chromatography), the lipid was redissolved in chloroform:methanol (1:1) and again evaporated to dryness. The dried lipid extract was redissolved in chloroform and separated by column chromatography on silicic acid and Florisil as described by Jose & Seale (1979) into phospholipid, neutral lipid and free fatty acid fractions. The phospholipid fraction was further separated into phosphatidyl

choline, phosphatidyl ethanolamine and other phosphatides by thin layer chromatography using the method of Cohen & Derekson (1969).

Radioactivity in column eluates was measured by evaporating the organic solvents to dryness, adding 2 ml. methanol and, after 1 hr at room temperature, adding 8 ml. scintillation fluid B (see below). Radioactivity on thin layer chromatography plates was measured by extracting the strip cut from the plate 1 cm wide or the gel scraped from glass-backed plates with 2 ml. methanol for 1 hr before adding 8 ml. scintillant B.

Liquid scintillation counting

Radioactivity was estimated in Packard Tricarb liquid scintillation counters. Counting efficiency was calculated from a sample channels ratio and all results were converted to d.p.m. The two scintillants used were: A: 5 g PPO, 0·25 g dimethyl POPOP, 1 l. toluene and 0.5 l. Triton X-100; B: 5 g PPO, 0·25 g dimethyl POPOP and 1 l. toluene.

Materials

Arachidonic acid, bovine serum albumin (fraction IV) and prostaglandin E_2 were obtained from Sigma. Indomethacin (Merck, Sharp & Dohme), diethylcarbamazine (Burroughs Wellcome), frusemide (Hoechst), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), hyoscine hydrobromide and propranolol hydrochloride (I.C.I.), phenoxybenzamine hydrochloride (Smith Kline & French) were generous gifts. Radioactive compounds, 1-[^{14}C]arachidonic acid (54 mc/m-mole) and [^{131}I]human serum albumin were obtained from the Radiochemical Centre, Amersham.

Differences between means were considered significant if $P < 0{\cdot}05$ as determined by the unpaired *t*-test.

RESULTS

Bio-assay of effluent

Following perfusion of arachidonic acid through guinea-pig lungs, biological activity appeared in the effluent contracting the rat stomach strip, rabbit aorta and rat colon. The same amounts of arachidonic acid perfused directly over the tissues produced no responses on any of the assay tissues. When the response of the rat stomach strip was calibrated in terms of prostaglandin E_2, injections of 1–5 μg of arachidonic acid into the lung produced about 20–100 ng prostaglandin E_2 equivalents. If we assume all this is due to conversion of infused arachidonic acid, guinea-pig lung converts $2 \pm 0{\cdot}2$ % (mean ± S.E. mean, n = 18) of infused arachidonic acid.

Recently, prostaglandin I_2 has been detected as a component of the myotropic products of arachidonic acid infusion through the pulmonary circulation of guinea-pig isolated lung (Alabaster & Hawkeswood, 1978). This is possible because the activity due to thromboxane A_2 and endoperoxides decays faster than that due to prostaglandin I_2. Using a delay coil (Alabaster & Hawkeswood, 1978), we were able to show in some experiments ($n = 6$) the presence of a rat colon relaxing substance when contractor activity on the rat stomach strip and rabbit aorta had largely disappeared. The relaxing substance was more readily detected at low levels of arachidonic acid (0·5–2 μg) as less interfering contractor substances were present at these concentrations.

Radiochemical assay of effluent

During and after infusion of [^{14}C]arachidonic acid (1·25 μg. ml^{-1}; 4 μM for 3 min) through guinea-pig lungs, the effluent was collected in 1 min fractions and the total

radioactivity in samples of these fractions measured (Fig. 1). Compared with the efflux of radioactivity following infusion of ^{131}I human serum albumin, that following [^{14}C]arachidonic acid attained its peak value later and fell more slowly. In three experiments, the total ^{14}C in lung effluent at 5 min, i.e. 2 min after the end of the infusion, was $17{\cdot}5 \pm 1{\cdot}5\%$ of the radioactivity infused. In most of our experiments we collected effluent for 10 min. At this time the effluent radioactivity was at a

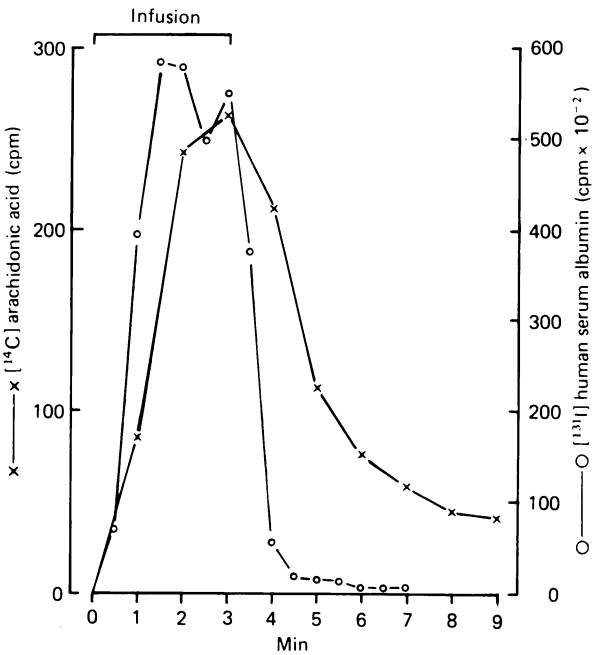

Fig. 1. Efflux of radioactivity from guinea-pig isolated lung following infusion of [^{14}C]arachidonic acid and [^{131}I]human serum albumin. The infusions were made into the pulmonary circulation for 3 min (horizontal bar) and effluent collected for the times shown, in 1 min fractions after infusion of arachidonic acid and 0·5 min after infusions of human serum albumin. Total radioactivity in 1 ml. samples of each fraction was measured and is shown on the vertical axes.

constant low level and the proportion of infused radioactivity in the effluent was $18 \pm 2\%$ (n = 15).

Radioactivity in the effluent collected up to 5 min and up to 10 min was analysed by thin layer chromatography after extraction. Several radioactive peaks were observed (Fig. 2) with most of the effluent radioactivity being associated with cyclo-oxygenase products (Table 1). Although we used prostaglandin $F_{2\alpha}$, E_2 and D_2 as markers for the cyclo-oxygenase product area, we did not seek to identify any particular peak in the area with any particular marker compound. Metabolism of arachidonic acid via cyclo-oxygenase was demonstrated as early as 5 min when the unchanged arachidonic acid and the hydroxy fatty acid components amounted to about one third of the total label collected. By 10 min cyclo-oxygenase products were clearly the major components with only about 10% unchanged arachidonic acid.

In two experiments, the infusion of [^{14}C]arachidonic acid was repeated after 30 min and the efflux of label followed as before. In each case, the proportion of infused

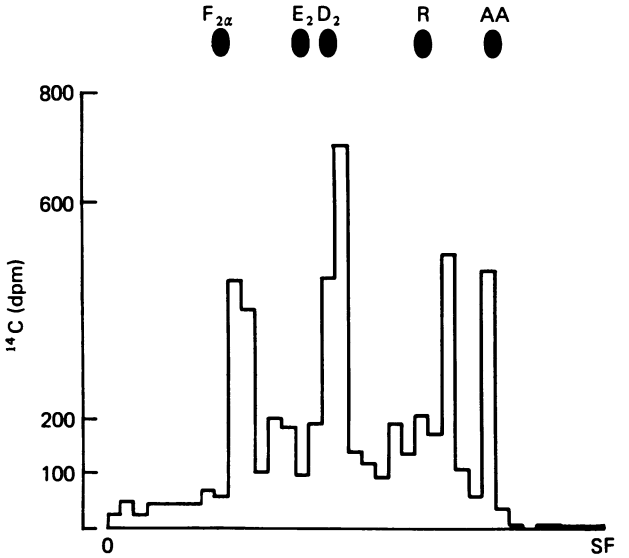

Fig. 2. Analysis of effluent from guinea-pig lung following infusion of [^{14}C]arachidonic acid. Effluent was collected for 10 min (3 min infusion plus 7 min further perfusion), acidified and extracted with diethyl ether. After evaporation the residue was separated on thin layers of silica gel with standards of prostaglandins $F_{2\alpha}$, E_2 and D_2, rincinoleic acid and arachidonic acid.

TABLE 1. Distribution of ^{14}C-label in effluent and lung after infusion of [^{14}C]arachidonic acid through the pulmonary circulation of guinea-pig isolated lungs

	Effluent (% total collected)			Lung (% total retained)		
	Cyclo-oxygenase metabolites	Hydroxy-fatty acids	Arachidonic acid	Phospho-lipid	Neutral lipid	Free acid
Control						
5 min (n = 3)	52 ± 5	13 ± 3	24 ± 7	78 ± 2	13 ± 2	6 ± 1
10 min (n = 15)	68 ± 3	14 ± 2	11 ± 2	72 ± 2	17 ± 2	7 ± 2
Albumin (1% BSA**)						
10 min (n = 3)	3 ± 1*	3 ± 1*	94 ± 1*	88 ± 2*	6 ± 1*	4 ± 3
Indomethacin (14 μM)						
10 min (n = 4)	25 ± 7*	7 ± 1*	56 ± 12*	68 ± 3	18 ± 1	10 ± 3
Diethylcarbamazine (3·8 mM)						
10 min (n = 5)	38†	18†	34†	68 ± 1	18 ± 1	8 ± 1
Bromcresol green (10 μM)						
10 min (n = 3)	39 ± 4*	5 ± 1*	40 ± 7*	64 ± 2*	23 ± 1	6 ± 1

The values in the Table are the means (± S.E. mean) from the numbers of experiments shown. Effluent was collected from the start of the infusion of [^{14}C]arachidonic acid and for a subsequent 2 min (5 min total) or for a subsequent 7 min (10 min total). At the end of the collection, the perfusion was stopped and the lungs removed from the perfusion chamber for analysis of retained radioactivity. Radioactivity in the effluent and in lung was analysed by t.l.c. and column chromatography as described in the Methods.

* Significantly different from corresponding value for 10 min control ($P < 0.05$; t test).

† These values are the mean of two experiments.

** Bovine serum albumin.

radioactivity emerging in the 10 min after infusion was similar to that after the first infusion with a mean value of 20% for the second infusion. In two experiments, infusion of [^{14}C]arachidonic acid was followed 20 min later by the same concentration of unlabelled arachidonic acid. There was no increase in the radioactivity in the effluent during or after the second infusion.

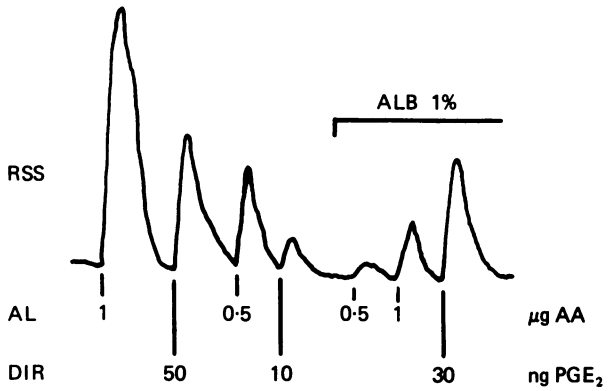

Fig. 3. Decreased biological activation of arachidonic acid in guinea-pig pulmonary circulation in the presence of bovine serum albumin. The Figure shows the responses of a rat stomach strip superfused with the effluent from a guinea-pig isolated lung. Injections of arachidonic acid made into the pulmonary circulation (AL) caused contractions of the rat stomach strip which were compared with contractions caused by injections of prostaglandin E_2 (PGE_2) given directly (DIR) to the assay tissue. On the left-hand side of the record 1 μg arachidonic acid given into the pulmonary circulation is more active than 50 ng PGE_2 and 0·5 μg arachidonic acid is equivalent to about 30 ng PGE_2. Although not shown in this Figure these amounts of arachidonic acid given directly to the rat stomach strip do not cause contractions of this tissue. When bovine serum albumin (ALB 1%) was added to the perfusate, 1 μg arachidonic acid given into the pulmonary circulation was much less active, approximately equivalent to 15 ng PGE_2. The presence of albumin did not affect the sensitivity of the rat stomach strip to PGE_2 given directly.

Radiochemical analysis of lung

The radioactivity retained in the lung at 5 and 10 min after the start of the infusion of arachidonic acid was separated into phospholipid, neutral lipid and fatty acid fractions (Table 1). At 5 min over 70% of the label was associated with phospholipid with less than 10% of free arachidonic acid and by 10 min, this distribution of label was not significantly changed. In five experiments, the labelled phospholipid fraction from lungs analysed at 10 min was further separated. The distribution of ^{14}C was: phosphatidyl choline 30 ± 3%; phosphatidyl ethanolamine 33 ± 2%; phosphatidyl inositol, phosphatidyl serine and sphingomyelin, 35 ± 3%.

Effect of albumin

In blood, arachidonic acid, like other free fatty acids, is extensively bound to plasma protein and we therefore added bovine serum albumin (final concentration of 1% w/v) to the Krebs perfusion medium in a series of experiments. The addition of bovine serum albumin decreased the biological activation of arachidonic acid as

illustrated by Fig. 3. In this experiment, biological activation of arachidonic acid was assayed on a rat stomach strip and the left hand part of the Figure shows that 1 μg arachidonic acid, after passage through the pulmonary circulation (AL) was converted to over 50 ng PGE_2 equiv., when the perfusate was plain Krebs solution. In the right-hand part, bovine serum albumin was added to the perfusate and now 1 μg arachidonic acid was equivalent to less than 30 ng prostaglandin E_2. To quantify

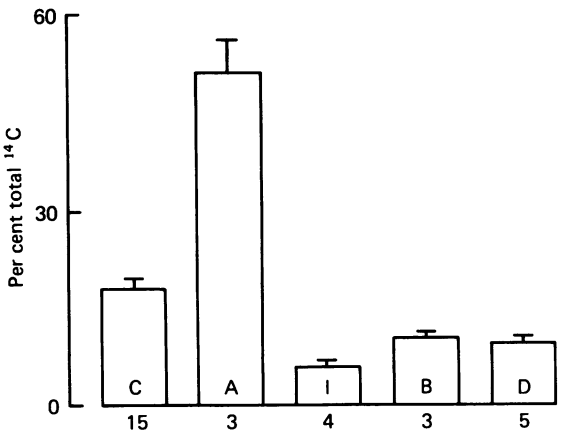

Fig. 4. Total radioactivity in effluent from guinea-pig lungs following [^{14}C]arachidonic acid infusions in different conditions. Effluent was collected for 10 min (3 min infusion + 7 min further perfusion) in a single fraction. The radioactivity in a sample of this combined effluent was measured and the total radioactivity in the whole 10 min fraction calculated. This value has been expressed as a percentage of the total infused and the heights of the bars represent this proportion (means ± S.E. means). The letters in the bars represent the various conditions – C, control; A, bovine serum albumin (1 %); I, indomethacin (14 μM); B, bromcresol green (10 μM); D, diethylcarbamazine (3·8 mM), and the numbers below each bar show the number of experiments in each condition.

this decreased activation, we calculated the dose ratio, i.e. the ratio of concentrations of infused arachidonic acid required in the same lung with and without bovine serum albumin to produce the same contraction on the rat stomach strip measured as ng PGE_2 equivalents. From six such experiments in guinea-pig lung the mean (± S.E.) dose ratio was 9 ± 1·5. Thus about nine times more arachidonic acid was needed in the presence of 1 % bovine serum albumin to produce as much activity in the effluent.

The addition of bovine serum albumin in the radioactive experiments caused a fall in radioactive cyclo-oxygenase products in the effluent, from about 70 to 3 %, with a corresponding rise in free arachidonic acid (Table 1). However, the proportion of infused radioactivity emerging in the effluent at 10 min increased to more than double the value in the absence of serum albumin (Fig. 4). The distribution of radioactivity in lung was affected by adding serum albumin to the perfusate during infusion of arachidonic acid, the neutral lipid pool being markedly decreased and the proportion in phospholipid increasing (Table 1).

Inhibition of cyclo-oxygenase activity

The presence of indomethacin in the Krebs perfusate markedly reduced the cyclo-oxygenase products in lung effluent following [^{14}C]arachidonic acid infusion (Table 1) and its biological activation in guinea-pig isolated lung. However, the total radioactivity in the effluent decreased to almost half the control value (Fig. 4). Analysis of radioactivity retained in the lung after 10 min showed no significant change from that observed in the absence of indomethacin (Table 1).

Three other drugs were observed to decrease biological activation of arachidonic acid, frusemide (1 mM), bromcresol green (10 μM) and diethylcarbamazine (3·8 mM). In terms of prostaglandin E_2 ng equivalents assayed on the rat stomach strip, this concentration of frusemide (eight experiments) decreased activation by 38 ± 6%, bromcresol green (eight experiments) decreased activation by 33 ± 7% and diethylcarbamazine (four experiments) decreased activation by 77 ± 7%. Two of these drugs, bromcresol green and diethylcarbamazine, were further studied with [^{14}C]arachidonic acid infusions. Both drugs decreased total effluent radioactivity (Fig. 4) but only bromcresol green had a small effect on the distribution of retained radioactivity (Table 1).

DISCUSSION

Our results show that there is an avid retention of arachidonic acid by the guinea-pig lung with less than a third of the total infused emerging in the effluent. Of this proportion more than half could be cyclo-oxygenase products. This term covers all endoperoxide-derived compounds except possibly the hydroxy acid HTT and includes thromboxanes, prostaglandins, prostacyclins and their degradation products. Because of the work of Hamberg *et al.* (1976), we were not concerned to analyse the radioactive products in any detail, but to try to correlate the amount of radioactive cyclo-oxygenase products with the amount of biological activity in the effluent. Our bio-assays showed about 2% conversion of infused arachidonic acid to myotropic metabolites. This value does not need correction for the effects of unchanged arachidonic acid as the amounts used did not affect the assay tissues and conversion thus represents only activity due to metabolites. The radiochemical assays showed about 14% (i.e. 70% of 20%) of infused arachidonic acid as cyclo-oxygenase products. Considering that PGI_2 is present in the effluent and has about 20% of the potency of PGE_2 on the rat stomach strip (Gryglewski, Bunting, Moncada, Flower & Vane, 1976), and that the endoperoxides and thromboxane A_2 are about half as potent as PGE_2 (Hamberg *et al.* 1976), we feel that these two assays are close enough to be able to conclude that the myotropic substances in lung effluent following infusion of arachidonic acid are derived from the exogenous substrate and not from endogenous substrate.

The slower appearance of the ^{14}C label from arachidonic acid compared with that of [^{131}I]human serum albumin suggests that arachidonic acid leaves the vascular compartment and is not metabolized solely by enzymes on the luminal surface of endothelial cells as is, for instance, angiotensin I (Ryan & Ryan, 1977). This is compatible with the suggestion (Gryglewski *et al.* 1976) that endothelial cells have only

prostacyclin synthetase and that thromboxane synthetase and cyclo-oxygenase is located elsewhere, perhaps in smooth muscle cells.

Our experiments also disclosed an avid uptake of label by the lung which did not reflect a simple binding by lung protein of free arachidonic acid as the majority of retained label appeared in phospholipid. Although in platelets (Bills, Smith & Silver, 1976; Blackwell, Duncombe, Flower, Parsons & Vane, 1977) and in spleen slices (Flower & Blackwell, 1976), exogenous radioactive arachidonic acid was also incorporated into phospholipid, our results differ in two respects. In platelets, the majority of label was associated with phosphatidyl choline, whereas we found a more uniform distribution of label. Also the rate of incorporation in lung is rapid compared, for instance, with spleen slices where after 10 min of incubation with [^{14}C] substrate, all the label taken up was associated with free arachidonic acid (Flower & Blackwell, 1976). In our work, as early as 5 min, over 70% of the label was associated with phospholipid. This rapid incorporation was not due to a high turnover rate of arachidonic acid containing phospholipid as in the next 5 min the label had the same distribution and, after 120 min only a further 10% of label had been lost in the effluent (Al-Ubaidi *et al.* 1978).

The avidity of the uptake of arachidonic acid described here is not organ specific as Isakson, Raz & Needleman (1976) observed a similar proportion of label retained in rabbit heart and kidney infused with [^{14}C]arachidonic acid, although they did use a longer perfusion time (20 min). They also found over 75% of retained label in phospholipid.

Addition of albumin to the perfusate decreased markedly the activation of exogenous arachidonic acid. This could be due either to a redirection of endoperoxide metabolism from thromboxane A_2 to prostaglandin I_2 or hydroxy acid HHT, both less potent contractors of the rat stomach strip, or to a decreased formation of endoperoxide. We favour the latter possibility as the effluent contained less cyclo-oxygenase products which would include PGI_2 break-down products and less hydroxy acid, pointing to a depressed synthesis of endoperoxide. Incorporation of label into lung phospholipids and other fractions was less affected, suggesting, as would be expected, that the lung was still capable of competing successfully with albumin for free fatty acids. A possible inference from these results is that, *in vivo*, binding of blood-borne arachidonic acid to albumin serves to direct arachidonic acid towards incorporation in tissue phospholipid and away from activation by cyclo-oxygenase into a number of highly potent products.

When cyclo-oxygenase activity was inhibited by drugs, for instance, indomethacin, we had expected the proportion of label in the effluent to remain constant but for the majority of it to be unchanged arachidonic acid. However, although the latter expectation was realized, the proportion of label in the effluent decreased after indomethacin. From these results it seems that at least half the substrate unused by cyclo-oxygenase was taken up by the alternative incorporation process. An equally unexpected finding was the inhibition of cyclo-oxygenase by three other drugs, none of which has previously been described as a cyclo-oxygenase inhibitor. Our purpose in using two of them, frusemide and bromcresol green, was to inhibit the inactivation of prostaglandins (Bito & Baroody, 1975; Hansen, 1976) formed from infused

arachidonic acid and thus to increase the amount of biological activity in the effluent. However, these drugs decreased the activation of the infused substrate and the radio-chemical analyses showed an effect qualitatively like indomethacin, i.e. decrease of effluent radioactivity and decrease in cyclo-oxygenase products. Either these drugs inhibit cyclo-oxygenase itself or they prevent uptake of substrate into cyclo-oxygenase-containing cells but not uptake leading to incorporation. Diethylcarbamazine inhibits the formation of SRS-A in rats (Orange, Valentine & Austen, 1968) and during anaphylaxis in guinea-pig lung, increases the formation of prostaglandin-like substances from endogenous arachidonic acid (Engineer, Niederhauser, Piper & Sirois, 1978). However, in our experiments, the formation of cyclo-oxygenase products from exogenous arachidonic acid was inhibited by this drug together with a decrease in the total radioactivity in the lung effluent, again suggesting an inhibition of the cyclo-oxygenase pathway but not of incorporation. None of the drugs studied had a marked effect on the distribution of label retained in the lung, implying that the various enzymes involved in the metabolism of glycerides and phosphoglycerides were little affected.

In summary, exogenous arachidonic acid infused through the pulmonary circulation of guinea-pig lungs is exposed to two metabolic processes. The major process entails uptake into lung and rapid incorporation into neutral and phospholipids and this arachidonic acid is retained firmly by the tissue. The minor process entails attack by cyclo-oxygenase and loss of arachidonic acid metabolites into the perfusate to appear in lung effluent. The primacy of the incorporation pathway is reinforced by the effects of albumin and by the effects of cyclo-oxygenase inhibition. It seems, therefore, that the major metabolites of exogenous arachidonic acid passing through the pulmonary circulation are phospholipid compounds and not the myotropic products derived via cyclo-oxygenase.

Dr Al-Ubaidi was supported by a Government of Iraq Postgraduate Scholarship. We are also very grateful to Dr P. Jose for his guidance with the analyses.

REFERENCES

ALABASTER, V. A. & HAWKESWOOD, E. (1978). PGI_2 release from guinea-pig lungs: detection and bioassay. *Br. J. Pharmac.* **62**, 467P.

AL-UBAIDI, F., BAKHLE, Y. S., JOSE, P. J. & SEALE, J. P. (1978). Fate of arachdonic acid in guinea-pig isolated lungs. *J. Physiol.* **281**, 2–3P.

BAKHLE, Y. S., REYNARD, A. M. & VANE, J. R. (1969). Metabolism of the angiotensins in isolated perfused tissues. *Nature, Lond.* **222**, 956–959.

BLACKWELL, G. J., DUNCOMBE, W. G., FLOWER, R. J., PARSONS, M. E. & VANE, J. R. (1977). The distribution and metabolism of arachidonic acid in rabbit platelets during aggregation and its modification by drugs. *Br. J. Pharmac.* **59**, 353–366.

BILLS, T. K., SMITH, J. B. & SILVER, M. J. (1976). Metabolism of [1^{14}C]arachidonic acid by human platelets. *Biochim. biophys. Acta* **241**, 303–314.

BITO, L. Z. & BAROODY, R. A. (1975). Inhibition of pulmonary prostaglandin metabolism by inhibitors of prostaglandin bio-transport (probenecid and bromocresol green). *Prostaglandins* **10**, 633–639.

COHEN, P. & DEREKSON, A. (1969). Comparison of phospholipid and fatty acid composition of human erythrocytes and platelets. *Br. J. Haemat.* **17**, 359–371.

ECKENFELS, A. & VANE, J. R. (1972). Prostaglandins, oxygen tension and smooth muscle tone. *Br. J. Pharmac.* **45**, 451–462.

ENGINEER, D. M., NIEDERHAUSER, U., PIPER, P. J. & SIROIS, P. (1978). Release of mediators of anaphylaxis; inhibition of prostaglandin synthesis and the modification of release of slow reacting substance of anaphylaxis and histamine. *Br. J. Pharmac.* **62**, 61–66.

FLOWER, R. J. & BLACKWELL, G. J. (1976). The importance of phospholipase A_2 in prostaglandin biosynthesis. *Biochem. Pharmac.* **25**, 285–291.

GRYGLEWSKI, R. J., BUNTING, S., MONCADA, S., FLOWER, R. J. & VANE, J. R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (Prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* **12**, 685–713.

HAMBERG, M., SVENSSON, J., HEDQVIST, P., STRANDBERG, K. & SAMUELSSON, B. (1976). Involvement of endoperoxides and thromboxanes in anaphylactic reactions. In *Advances in Prostaglandin and Thromboxane Research*, vol. **1**, ed. SAMUELSSON, B. & PAOLETTI, R., pp. 495–501. New York: Raven.

HANSEN, H. S. (1976). 15-Hydroxyprostaglandin dehydrogenase – a review. *Prostaglandins* **12**, 647–679.

ISAKSON, P. C., RAZ, A. & NEEDLEMAN, P. (1976). Selective incorporation of ^{14}C-arachidonic acid into the phospholipids of intact tissues and subsequent metabolism to ^{14}C-prostaglandins. *Prostaglandins* **12**, 739–748.

JOSE, P. J. & SEALE, J. P. (1979). Incorporation and metabolism of ^{14}C-arachidonic acid in guinea-pig lungs. *Br. J. Pharmac.* (in the Press).

ORANGE, R. P., VALENTINE, M. D. & AUSTEN, K. F. (1968). Inhibition of the release of slow reacting substance of anaphylaxis in the rat with diethylcarbamazine. *Proc. Soc. exp. Biol. Med.* **127**, 127–132.

PALMER, M. A., PIPER, P. J. & VANE, J. R. (1973). Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical or mechanical stimulation of guinea-pig lungs. *Br. J. Pharmac.* **49**, 226–242.

RYAN, J. W. & RYAN, U. S. (1977). Pulmonary endothelial cells. *Fedn Proc.* **36**, 2683–2691.

VANE, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.* **23**, 360–373.