Biochem. J. (1984) **222**, 657–662
Printed in Great Britain

Arachidonyl transfer from diacyl phosphatidylcholine to ether phospholipids in rat platelets

Odile COLARD, Michelyne BRETON and Gilbert BEREZIAT
Laboratoire de Biochimie, CHU Saint Antoine, 27 rue Chaligny, 75571 Paris Cedex 12, France

(*Received 19 March 1984/Acepted 29 May 1984*)

High levels of ether phospholipids were found in rat platelets. Alkylacyl compounds constituted 18 and 29% of glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE). Alkenylacyl compounds, not detected in GPC, represented 40% of GPE. Arachidonate comprised 60%, 42% and 26% of the acyl residues in the *sn*-2 position of alkenylacyl-GPE, alkylacyl-GPE and alkylacyl-GPC respectively. Based on all arachidonate being linked to the *sn*-2 position of the diacyl species, the arachidonate level was 47% in diacyl-GPE and 30% in diacyl-GPC. The incorporation and metabolic fate of arachidonate in various phospholipid classes of resting platelets was examined. Arachidonate was essentially recovered in the diacyl phospholipids and very poorly in alkylacyl- and alkenylacyl-GPE and GPC after 30 min incubation in the presence of [^{14}C]arachidonic acid. Upon reincubation of the platelets after removal of free arachidonate, the radioactivity was gradually lost from diacyl-GPC. Concomitantly, the radioactivities in alkylacyl-GPC, alkylacyl-GPE, alkenylacyl-GPE and to a lower extent in diacyl-GPE were increased. Labelling of glycerophosphoinositol was not changed. This labelling transfer was linear up to 5–6 h, except for alkylacyl-GPC; then labelling remained constant. These data strongly suggest that free arachidonate incorporation through the Lands pathway occurs only for diacyl species and that arachidonate incorporation into the ether phospholipids is achieved by exchange from diacyl-GPC. Based on specific activities related to phosphorus content, the arachidonate incorporation rates into diacyl-GPE and diacyl-GPC were approximately equivalent. The very large differences between specific radioactivities related to arachidonate observed at the starting reincubation time were strongly attenuated when labelling equilibrium was reached. The turnover rate by this exchange pathway was higher in alkylacyl-GPC than in alkyl- and alkenylacyl-GPE. This finding agrees with the selectivity for arachidonate observed in the acylation of PAF-acether in human neutrophils [Chilton, O'Flaherty, Ellis, Swendsen & Wykle (1983) *J. Biol. Chem.* **258**, 7268–7271].

Discrepancies between platelet phospholipid arachidonate content and [^{14}C]arachidonic acid incorporation into platelet phospholipids have been recognized for a long time (Cohen & Derksen, 1969; Bills *et al.*, 1977). Alkenylacyl-GPE, which contains about 60% of arachidonate (Cohen & Derksen, 1969), was particularly poorly labelled. Radioactive arachidonate transfer from platelet phospholipids to alkenyl-GPE was observed when platelets were stimulated with thrombin (Rittenhouse-Simmons *et al.*, 1977) or ionophore (Vargaftig *et al.*, 1980). A possible arachidonyl exchange into ether phospholipids was also indicated by studies on rat testicular tissues (Blank *et al.*, 1973)

In a previous study (Colard *et al.*, 1984) we have demonstrated a CoA-mediated specific arachidonyl transfer from prelabelled platelet phospholipids to various added lysophospholipids in rat platelet homogenates. Kramer & Deykin (1983) also observed an arachidonyl transfer from GPC to alkenyl-GPE in human platelet homogenates. As this exchange mechanism could explain the arachidonate enrichment of certain phospholipids, we

Abbreviations used: GPC, glycero-3-phosphocholine; GPE, glycero-3-phosphoethanolamine; GPI, glycero-3-phosphoinositol; GPS, glycero-3-phosphoserine; GPL, glycerophospholipid; PAF, platelet-activating factor.

investigated arachidonate movements in resting intact platelets and found a transfer from GPC to GPE.

High levels of alkylacyl-GPC and -GPE as well as alkenylacyl-GPC and -GPE were recently found in rabbit (Sugiura *et al.*, 1983) and human (Mueller *et al.*, 1983) platelets. Whereas alkenylacyl compounds are easily dissociated from the related diacyl phospholipids by acidic hydrolysis, it is more difficult to obtain the alkylacyl compounds free from the diacyl compounds. These difficulties have impeded studies on arachidonate turnover. However, considerable interest has been focused on alkylacyl-GPC, since the structure of PAF-acether has been shown to be 1-alkyl-2-acetyl-GPC (Demopoulos *et al.* 1979; Benveniste *et al.*, 1979). PAF-acether would be synthesized from alkylacyl-GPC by phospholipase A_2 hydrolysis followed by an acetylation (Wykle *et al.*, 1980; Ninio *et al.*, 1982; Mueller *et al.*, 1982). Conversely, PAF-acether inactivation could occur in platelets and neutrophils through deacetylation followed by acylation into alkylacyl-GPC (Chilton *et al.*, 1982; Touqui *et al.*, 1983; Pieroni & Hanahan, 1983). In this inactivating acylation reaction, a selectivity for arachidonate was observed in human neutrophils (Chilton *et al.*, 1983)

It was, therefore, of interest to investigate arachidonate changes in alkenylacyl and diacyl, as well as in alkylacyl, phospholipids. Using a new method described by El Tamer *et al.* (1984) for complete ether phospholipid analysis, we found that a long-term arachidonyl transfer from diacyl-GPC to alkylacyl-GPC, alkylacyl-GPE, alkenylacyl-GPE and to a lesser extent diacyl-GPE occurred in resting rat platelets.

Materials and methods

Guinea pig pancreas phospholipase A_1 was a gift from Dr. J. Fauvel, who identified and purified this enzyme (Fauvel *et al.*, 1981). The fraction used was the cationic lipase I. The enzyme (250i.u./ml) was stored at −20°C in 50mM-Tris/HCl buffer (pH9.0) containing 50% (v/v) glycerol. [1-^{14}C]Arachidonic acid (59Ci/mol) was purchased from The Radiochemical Centre, Amersham. Silica gel G-coated plates were obtained from Merck or from Schleicher and Schull. Fatty-acid-free albumin and acetylsalicylic acid were obtained from Sigma Chemical Co. Gelatin was from Merck.

Platelets

Platelet-rich plasma was prepared by centrifugation at 375*g* for 7min of citrated blood from Wistar rats. Platelets were then isolated by centrifugation at 1400*g* for 15min at 30°C and resuspended in the same volume of buffer, pH6.5, containing 2.6mM-KCl, 1mM-$MgCl_2$, 137mM-NaCl, 12mM-$NaHCO_3$, 0.2mM-EGTA, 5.5mM-glucose and 0.25% gelatin (Ardlie *et al.*, 1970). After 30min at room temperature in the presence of 0.1mM-acetylsalicylic acid, this platelet suspension was centrifuged at 1400*g* for 10min and the resulting pellet resuspended in the same buffer. The suspension was adjusted to 1×10^9 platelets/ml.

Platelets were labelled by incubating the platelet suspension with [1-^{14}C]arachidonic acid (0.8μCi/ml of platelet suspension) and 0.25% fatty acid-free albumin for 30min at 37°C. The platelets were then pelleted, resuspended and centrifuged twice more to remove contaminating arachidonic acid. Platelet samples taken from the last resuspension were considered as time 0. The remaining platelet suspension was reincubated at 37°C for various lengths of time. Aggregation response to 0.4 unit of thrombin/ml was assayed at the various reincubation times tested. Aggregation reached a maximum at 3min at time 0 and after 2h of reincubation. Maximum aggregation response of platelets after 5h of reincubation was 70% compared with that at time 0. After 18h of reincubation, the platelets did not aggregate.

Lipids

Platelet lipids were extracted by the method of Folch *et al.* (1957). Individual phospholipids were separated by two-dimensional t.l.c. using silica-gel coated plates obtained from Merck and solvent systems I [chloroform/methanol/acetic acid/water (25:15:4:2, by vol.] and II (tetrahydrofuran/methylal/methanol/water (10:5:5:1, by vol.)]. Lipids spots were detected by exposure to iodine vapour. After evaporation of iodine, the phospholipids were scraped off the plate and the powder was directly assayed for lipid phosphorus by the method of Bartlett (1959) or counted in a liquid-scintillation counter.

Separation of the various classes of choline- and ethanolamine-containing phosphoglycerides was effected by the method of El Tamer *et al.* (1984). This method was based on the selective destruction of diacylphospholipids by guinea pig phospholipase A_1 and of alkenylacylphospholipids by acidolysis. The efficiency and reliability of this method for ether phospholipid analysis was clearly established by the extensive analysis recently performed by El Tamer *et al.* (1984) and Diagne *et al.* (1984). In practice, lipid extract corresponding to 1×10^9 platelets was dried under N_2, 1ml of 0.2M-Tris/HCl, pH8.0, containing 2.4mM-sodium deoxycholate was added and this mixture was sonicated twice for 15s. Phospholipase A_1 (0.5i.u.) was added and the mixture was incubated at 37°C for 60min. The reaction was stopped and extracted by

the method of Folch *et al.* (1957). The lipid extract was dried, then spotted onto a t.l.c. plate and developed for 50min in the solvent system chloroform/methanol/acetic acid/water (180:108:11:11, by vol.). After drying, the plate was subjected to HCl fumes for 10min and left to evaporate for at least 1h before developing in a second direction with chloroform/methanol/14M-NH_3/water (180:108:11:11, by vol.). The various phospholipids were visualized by exposure to iodine vapour or by autoradiography. Sphingomyelin, which was not hydrolysed, was taken as an internal standard for phospholipid phosphorus assay.

Fatty acids were analysed as the methyl esters by g.l.c. on a Girdel 30 chromatograph. The temperature of the CP Wax capillary column was 210°C. Ethanolamine- and choline-containing glycerophospholipids were isolated by t.l.c., then subjected separately to the phospholipase A_1 and HCl hydrolysis. The various spots were scraped off the plate and the lipids treated and analysed the same day. The fatty acid composition at position 2 of the ether phospholipids was obtained by mild alkaline hydrolysis and BF_3-catalysed methylation from alkylacyl-GPE and from HCl-hydrolysed acyl-GPE. Fatty acids at position 2 of the diacyl species were calculated by multiplying by 2 the percentage of the methyl esters obtained in mixing the phospholipase A_1-hydrolysed acyl-GPE and the fatty acid spot.

Results and discussion

Lipid phosphorus was estimated to be 230 ± 46nmol/10^9 rat platelets. The major phospholipid species were GPC and GPE. Thus GPE appeared to be somewhat higher than in human platelets (22.9%; Lagarde *et al.*, 1982) but was close to that in rabbit platelets (32.5%; Andreoli, 1968).

Table 1 shows the distribution of radioactivity in the phospholipids upon reincubation of [^{14}C]-arachidonate-labelled platelets. At time 0, radioactivity in GPC was 3–4 times higher than in GPE. Upon reincubation, the radioactivity in GPE gradually increased with time whereas that in GPC concomitantly decreased. The radioactivity recovered in the neutral lipid fraction was essentially incorporated into triacylglycerols and also decreased to some extent. The small increase in GPS labelling was not significant. The lack of change in GPI disagrees with the arachidonate transfer observed form GPC to GPI when platelets were incubated with 1-acyl-2-[^{14}C]arachidonyl-GPC-loaded high-density lipoprotein (Bereziat *et al.*, 1978). Thus there was a redistribution of [^{14}C]-arachidonate between phospholipids, essentially from GPC to GPE. Our assays on rat platelet homogenates (Colard *et al.*, 1984) have shown that an arachidonate transfer could be induced by a number of lysoderivatives, including 2-lysoGPC, 2-lysoGPE and 2-lysoGPS. When lysoGPE and -GPS were the acceptors, arachidonate came from GPC and when lysoGPC was the acceptor, the phospholipid donor was GPI. However, in whole rat platelet, as in rabbit macrophages (Sugiura *et al.*, 1984), no transfer from GPI or GPE occurred. The arachidonate exchange was essentially from GPC to GPE.

To investigate the role of diacyl and ether phospholipids in this arachidonate transfer, we hydrolysed the diacyl phospholipids by pancreatic phospholipase A_1 and the alkenyl phospholipids by HCl fumes in order to separate alkyl, alkenyl and diacyl compounds. The ether and diacyl composition of rat platelet choline- and ethanolamine-containing phospholipids (Table 2) was quite different from that of human (Mueller *et al.*, 1983) and rabbit (Sugiura *et al.*, 1983) platelets. Alkenylacyl-GPC was not detected in rat platelets (against 9% and 3% in human and rabbit) and considerable amounts of alkylacyl compounds were found in rat platelet GPE (29%, against 3% and 2%

Table 1. *Distribution of* [^{14}C]*arachidonate in the lipids upon reincubation*

Platelets were prelabelled with [^{14}C]arachidonic acid for 30min, then pelleted and washed twice with Tyrode buffer, pH6.5. Then the cells were again incubated at 37°C in the same buffer. Phospholipids were separated as described in the Materials and methods section. Neutral lipids (NL) were taken at the solvent front. The data are the mean ± S.E.M. of *n* determinations. Values in parentheses are the amounts (%) of the various phospholipids calculated from phosphorus assay (mean of four determinations).

	Distribution (%) at time:			
Lipids	0 ($n=6$)	60min ($n=3$)	120min ($n=5$)	240min ($n=2$)
GPC (37.1)	47.0±1.6	43.7±0.7	39.2±1.4	30.1±0.5
GPI (5.6)	20.2±2.1	21.9±0.1	20.0±1.6	18.6±1.6
GPS (11.1)	5.1±1.0	6.1±1.0	7.1±1.6	6.6±1.5
GPE (31.0)	12.7±1.2	15.0±0.5	19.3±1.8	22.2±2.5
NL	14.8±1.9	11.8±0.3	9.9±1.4	8.4±0.8

Table 2. *Composition of choline- and ethanolamine-containing GPL and their content of arachidonic acid*
The various fractions were separated by enzymic and acidic hydrolysis followed by two-dimensional t.l.c. as described in the Materials and methods section. The phospholipid values were calculated from the lipid phosphorus and the percentages of arachidonate were determined by g.l.c. The data are the mean ± S.E.M. of three separate determinations.

Component	GPL (nmol/10^9 platelets)	Arachidonate at position 2 (%)	Arachidonate in GPL (nmol/10^9 platelets)
Diacyl-GPE	19.4±3.2	47.4±2.3	9.2
Alkenylacyl-GPE	31.6±2.7	60.1±3.2	19.0
Alkylacyl-GPE	20.9±4.0	42.2±4.7	8.8
Diacyl-GPC	69.7±2.6	30.0±3.8	20.9
Alkylacyl-GPC	15.3±2.3	26.4±2.9	4.0

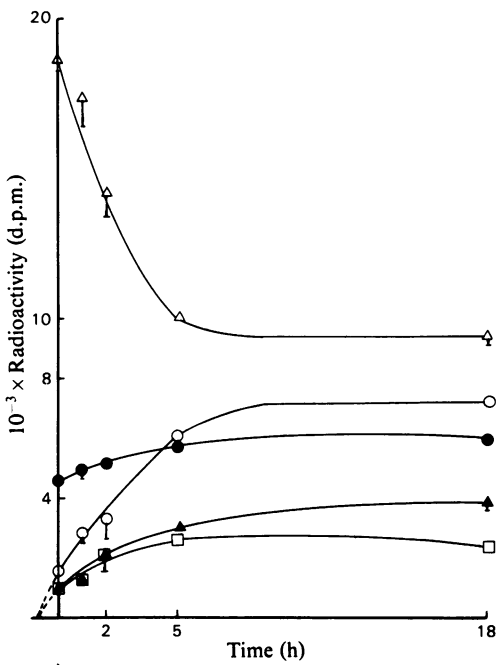

Fig. 1. *Time course of [^{14}C]arachidonyl transfer from PC to alkyl-PC and to alkyl-, alkenyl- and diacyl-PE*
Incubation conditions were as in Table 1. Phospholipid separation is described in the Materials and methods section. Data are the mean ± S.D. of two different determinations. △, Diacyl-GPC; □, alkylacyl-GPC; ●, diacyl-GPE; ○, alkenylacyl-GPE; ▲, alkylacyl-GPE.

in human and rabbit). The arachidonate content was the highest in alkenylacyl-GPE. It remained high in the diacyl and alkylacyl-GPE and was lower in the diacyl and alkylacyl-GPC. These results were close to those obtained on other platelets (Sugiura *et al.*, 1983; Mueller *et al.*, 1983), except for a higher archidonate content in alkylacyl-GPE in rat platelets than in human platelets. In any case, the amount of this phospholipid is very low in human platelets.

As shown in Fig. 1, the ethanolamine- as well as choline-containing ether phospholipids were poorly labelled at the start of the reincubation and the radioactivity was essentially recovered in diacyl compounds. As a function of time, the labelling of alkylacyl-GPC and -GPE and of alkenylacyl-GPE markedly increased, concomitantly with a decrease of diacyl-GPC labelling. A small increase of diacyl-GPE was also observed. These changes occurred in 5–6h, and then labelling was constant.

In extrapolating the curves of [^{14}C]arachidonate increases in the ether phospholipids, one can observe that the intersection with the abscissa is at about 60min before the start of reincubation. This period approximately corresponds to the time necessary for the platelet labelling and washing manipulation. This suggests that free [^{14}C]-arachidonate incorporation via the Lands pathway (Hill & Lands, 1968) occurs only in the diacyl species and that the ether phospholipid labelling is achieved by exchange from GPC, this exchange beginning as soon as [^{14}C]arachidonate is acylated into diacyl-GPC. The absence of ether phospholipid acylation from free arachidonate agrees with the much lower acyltransferase activities to alkenyl-GPE and to alkyl-GPC than to acyl lysoderivatives demonstrated in several tissues (Waku & Nakazawa, 1970; Wykle *et al.*, 1973; Natarajan & Sastry, 1973).

If this assumption is true, we can consider that the radioactivity recovered in the ether phospholipids at the start of reincubation was primarily incorporated into diacyl-GPC. This would increase the diacyl-GPC specific radioactivity related to phosphorus content (Table 3) from 1050 to 1250d.p.m./nmol. These values are not very different from the diacyl-GPE specific radioactivity. Thus the arachidonate incorporation rate into diacyl-GPC and diacyl-GPE by the Lands pathway would be approximately equivalent and the

Table 3. *^{14}C specific radioactivities related to the whole GPL and to GPL molecular species containing arachidonic acid*
[^{14}C]Arachidonate incorporation values were taken from Fig 1. The amounts of the various GPE and GPC classes and their content in arachidonate were taken from Table 2.

Component	Specific radioactivity (d.p.m./nmol)					
	Whole GPL			GPL containing arachidonate		
Time ...	0h	2h	18h	0h	2h	18h
Diacyl-GPE	920	1067	1256	1940	2250	2648
Alkenylacyl-GPE	195	421	917	324	700	1525
Alkylacyl-GPE	196	399	758	465	498	1800
Diacyl-GPC	1053	798	548	3512	2661	1827
Alkylacyl-GPC	315	537	606	1205	2054	2318

lower labelling of platelet GPE compared with GPC observed in short-term incubation (Rittenhouse-Simmons & Deykin, 1981) would be due to different levels in diacyl species.

If we consider the specific radioactivities related to arachidonyl content in each phospholipid class (Table 3), we observe that free arachidonate incorporation rates into the GPE and GPC diacyl species were different and consequently not related to the arachidonyl content. The specific radioactivity of arachidonate in diacyl-GPC was 3500d.p.m./nmol at the start of reincubation and arachidonate transfer to ether phospholipids was linear, allowing estimation of transfer rates. Arachidonate transfer rates are 2nmol/h per 10^9 platelets from diacyl-GPC and 1, 0.6 and 0.45nmol/h per 10^9 platelets respectively into alkenylacyl-GPE, alkylacyl-GPE and alkylacyl-GPC. Since arachidonyl transfer from diacyl-GPC is linear up to 5h, when equilibrium was reached, 10nmol of arachidonate, relative to a content of 21nmol in diacyl-GPC, have been exchanged.

It is obvious from these data that the pronounced differences between arachidonate specific radioactivities in the various phospholipids observed at the start of reincubation tended to decrease upon reincubation up to an equilibrium state. However, alkylacyl-GPC reached this equilibrium more rapidly than the other ether phospholipids. First, alkylacyl-GPC specific radioactivity was already higher than ether GPE specific radioactivities at the start of reincubation. This suggests that an arachidonyl transfer, more rapid to alkylacyl-GPC than to ether GPE, has occurred during arachidonic acid incorporation and washing of platelets. Second, the increase of alkylacyl-GPC specific radioactivity was almost complete after 2h reincubation, in contrast with the ethers and diacyl-GPE which increased linearly up to 5h. So it appears that arachidonic acid turnover by this exchange pathway was more rapid in alkylacyl-GPC than in the other phospholipids. This result agree with the striking selectivity for arachidonate observed by Chilton *et al.* (1983) in the acylation of deacetylated PAF-acether by human neutrophils.

We (Colard *et al.*, 1984) have found that CoA was a required factor in rat platelet homogenates for exchange of arachidonate from GPC to acyl-lysoGPE, whereas in human platelet homogenates, Kramer & Deykin (1983) observed a CoA-independent arachidonate transfer from GPC to alkenyl-lysoGPE. Perhaps different transacylation mechanisms, CoA-dependent and -independent, are involved in the specific transfer of arachidonate to the various alkyl, alkenyl and acyl compounds. In any case, lyso compounds were necessary for arachidonyl exchange between phospholipids in rat (Colard *et al.*, 1984) as in human (Kramer & Deykin, 1983) platelet homogenates. So this exchange is likely to be regulated by the production of lysoderivatives through a phospholipase A_2. It could explain the specific acylation by arachidonate of added l-alkyl-lysoGPC (lysoPAF) as well as l-acyl-lysoGPC and PAF-acether, which is rapidly deacetylated, into lysoPAF, observed in human neutrophils (Chilton *et al.*, 1983).

Further studies are required to determine if the transfer observed here is induced by the availability of ether lysophospholipids, and what is the role of arachidonyl transfer to the ether phospholipids in the release of biologically active metabolites.

This work was supported by INSERM grants 81.7006 and 131.002.

References

Andreoli, V. M. (1968) *Eur. J. Pharmacol.* **7**, 314–318
Ardlie, M. G., Packman, M. A. & Mustard, J. F. (1970) *Br. J. Haematol.* **19**, 7–17
Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
Benveniste, J., Tence, M., Varenne, P., Bidault, J., Boullet, C. & Polonsky, J. (1979) *C. R. Acad. Sci. Paris* **289**, 1037–1040

Bereziat, G., Chambaz, J., Trugnan, G., Pepin, D. & Polonovski, J. (1978) *J. Lipid Res.* **19**, 495–500
Bills, T. K., Smith, J. B. & Silver, M. J. (1977) *J. Clin. Invest.* **60**, 1–6
Blank, M. L., Wykle, R. L. & Snyder (1973) *Biochim. Biophys. Acta* **316**, 28–34
Chilton, F. M., O'Flaherty, J. T., Ellis, J. M., Swendsen, C. L. & Wykle, R. L. (1982) *J. Biol. Chem.* **258**, 6357–6361
Chilton, F. M., O'Flaherty, J. T., Ellis, J. M., Swendsen, C. L. & Wykle, R. L. (1983) *J. Biol. Chem.* **258**, 7268–7271
Cohen, P. & Derksen, A. (1969) *Br. J. Haematol.* **17**, 359–371
Colard, O., Breton, M. & Bereziat, G. (1984) *Biochim. Biophys. Acta* **793**, 42–48
Demopoulos, C. A., Pinckard, R. N. & Hanahan, D. J. (1979) *J. Biol. Chem.* **254**, 9355–0358
Diagne, A., Fauvel, J., Record, M., Chap, H. and Douste-Blazy, L. (1984) *Biochim. Biophys. Acta* **793**, 221–231
El Tamer, A., Record, M., Fauvel, J., Chap, H. & Douste-Blazy, L. (1984) *Biochim. Biophys. Acta* **793**, 213–220
Fauvel, J., Bonnefis, M. J., Sarda, L., Chap, H., Thouvenot, J. P. & Douste-Blazy, L. (1981) *Biochim. Biophys. Acta* **663**, 446–456
Folch, J., Lees, M. & Sloane-Stanley, G. M. (1957) *J. Biol. Chem.* **226**, 497–509
Hill, E. E. & Lands, W. E. M. (1968) *Biochim. Biophys. Acta* **152**, 645–654
Kramer, R. M. & Deykin, D. (1983) *J. Biol. Chem.* **258**, 13806–13811
Lagarde, M., Guichardant, M., Menashi, S. & Crawford, N. (1982) *J. Biol. Chem.* **257**, 3100–3104
Mueller, M. W., O'Flaherty, J. T. & Wykle, R. L. (1982) *J. Biol. Chem.* **258**, 6213–6218
Mueller, H. W., Purdon, A. D., Smith, J. B. & Wykle, R. (1983) *Lipids* **18**, 814–818
Natarajan, V. & Sastry, P. S. (1973) *FEBS Lett.* **32**, 9–12
Ninio, E., Mencia-Huerta, J. M., Heymans, F. & Benveniste, J. (1982) *Biochim. Biophys. Acta*, **710**, 23–31
Pieroni, G. & Hanahan, D. J. (1983) *Arch. Biochem. Biophys.* **224**, 485–493
Rittenhouse-Simmons, S., Russel, F. A. & Deykin, D. (1977) *Biochim. Biophys. Acta* **488**, 370–380
Rittenhouse-Simmons, S. & Deykin, D. (1981) in *Platelets in Biology and Pathology 2* (Gordon, J. L., ed.), pp. 349–372, Elsevier/North-Holland, Amsterdam
Sugiura, T., Soga, N., Nitta, M. & Waku, K. (1983) *J. Biochem. (Tokyo)* **94**, 1719–1722
Sugiura, T., Osamu, K., Fukui, J., Nakagawa, Y. & Waku, K. (1984) *FEBS Lett.* **165**, 273–276
Touqui, L., Jacquemin, C. & Vargaftig, B. B. (1983) *Biochem. Biophys. Res. Commun.* **110**, 890–893
Vargaftig, B. B., Fouque, F. & Chignard, M. (1980) *Thromb. Res.* **17**, 91–102
Waku, K. & Nakazawa, Y. (1970) *J. Biochem. (Tokyo)* **68**, 459–466
Wykle, R. L., Blank, M. L. & Snyder, F. (1973) *Biochim. Biophys. Acta* **326**, 26–33
Wykle, R. L., Malone, B. & Snyder, F. (1980) *J. Biol. Chem.* **255**, 10256–10260