Identification and characterization of two phospholipase A₂ activities in resident mouse peritoneal macrophages

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Resident mouse peritoneal macrophages synthesize and release large amounts of prostaglandins in response to inflammatory stimuli. Release of prostaglandin E₂ and 6-oxoprostaglandin F_{1α} occurs at a rate of 1 nmol/h per mg of cell protein. The mechanisms by which substrate arachidonic acid is released have yet to be established. We have therefore initiated studies to characterize those enzymes that can catalyse its release from phospholipid and may be of significance at the cellular level. We report initially the characterization of two phospholipase A₂ activities in homogenates of mouse peritoneal macrophages. The first is active at pH 4.5 and is not dependent on Ca²⁺. The second is Ca²⁺-dependent and is optimally active at pH 8.5. Either phospholipase A₂ activity is capable of hydrolysing [¹⁴C]arachidonic acid from [¹⁴C]arachidonic acid-labelled phospholipids in quantities sufficient to account for the amounts of prostaglandins released by macrophages in culture. Phospholipid substrates are prepared from mouse LM fibroblasts maintained in serum-free Higuchi medium containing radiolabelled phospholipid precursors. Single-labelled phospholipids bear the ¹⁴C label in the arachidonic acid moiety. Dual-labelled phospholipids bear a ¹⁴C label in the polar head group and a ³H label in the arachidonic acid moiety. Experiments with dual-labelled substrates establish that both phospholipase activities are of the A₂ type as indicated by the equimolar recovery of [³H]arachidonic acid and [¹⁴C]lysophospholipid. Studies with aqueous sonicated dispersions of purified [¹⁴C]arachidonic acid-labelled phospholipid or mixed liposomal substrates formed from mixtures of cellular polar lipids reveal that the pH 4.5 activity hydrolyses phosphatidylethanolamine and phosphatidylcholine more efficiently when they are present in a mixture of other polar lipids. The pH 8.5 activity, however, hydrolyses the purified phospholipids more efficiently.

We have reported that resident mouse peritoneal macrophages synthesize and release large quantities of oxygenated products of arachidonic acid when stimulated by exposure to inflammatory substances. Macrophages, exposed to substances such as zymosan (Humes *et al.*, 1977; Bonney *et al.*, 1978), phorbol myristate acetate (Humes *et al.*, 1978; Brune *et al.*, 1978) and antigen–antibody complexes (Bonney *et al.*, 1979a), release, as the major products of arachidonic acid oxygenation, prostaglandin E₂ and 6-oxoprostaglandin F_{1α} at an approximate rate of 1 nmol/h/mg of cellular protein (Bonney *et al.*, 1978). Reports originating from other investigators indicate that arachidonic acid constitutes 20% of the fatty acids associated with the phospholipids of mononuclear phagocytes (Mason

et al., 1972; Stossel *et al.*, 1974). However, the concentration of the non-esterified fatty acid is very low. Similarly, prostaglandins are not stored intracellularly but are synthesized and released rapidly in response to inflammatory stimuli (Kimze & Vogt, 1971). This synthesis is therefore limited by the availability of precursor arachidonic acid, which is normally not found in significant quantity except as a constituent of complex lipids, primarily phospholipids.

Other investigators have described lipolytic enzymes associated with tissues known to release prostaglandins on appropriate stimulation. Numerous useful reviews concerning this subject have been published (Coatt & Berenholz, 1973; van den Boosch, 1974; Lands & Crawford, 1976).

Although there is evidence that in special cases non-esterified arachidonic acid is derived from sources other than phospholipid (Zwaal *et al.*, 1973; Chap & Douste-Blazy, 1974), a vast body of evidence indicates that in most cases phospholipid is the source of prostaglandin precursor arachidonic acid. Franson & Waite (1973) have reported on a single lysosomal phospholipase A₂ from both bacillus Calmette-Guérin-induced and normal rabbit alveolar macrophages.

The capacity of resident mouse peritoneal macrophages to release prostaglandins on exposure to an inflammatory stimulus and the nature of this release have been well characterized in this laboratory (Humes *et al.*, 1977, 1978; Bonney *et al.*, 1978, 1979a). We have therefore undertaken the study of the various macrophage lipolytic enzymes that could play a role in these events. We use substrates that are relevant to the inquiry, [¹⁴C]arachidonic acid-labelled phospholipid, thus allowing for the quantification of the release of the prostaglandin precursor arachidonic acid. In the present paper we describe two phospholipase A₂ activities, each with sufficient catalytic capacity to supply non-esterified arachidonic acid in quantities adequate to account for the amounts of prostaglandins released by macrophages in culture.

Materials

Male Swiss Webster mice (HLA-SW/ICR SPF) were purchased from Hilltop Lab Animals, Scottsdale, PA, U.S.A. M199 medium, components for Higuchi medium and pig serum were purchased from GIBCO Inc., Grand Island, NY, U.S.A. The pig serum was inactivated by heating at 56°C for 30 min. Nunclon tissue-culture dishes were from Vangard International, Neptune, NJ, U.S.A. Tissue-culture flasks were purchased from Corning Glassworks, Corning, NY, U.S.A. [1-¹⁴C]Arachidonic acid (sp. radioactivity 51 Ci/mol), [5,6,8,9,11,12,14,15(n)-³H]arachidonic acid (sp. radioactivity 64 Ci/mmol), [1,2-¹⁴C]choline chloride (sp. radioactivity 6.4 Ci/mol), [1,2-¹⁴C]ethanolamine hydrochloride (sp. radioactivity 3.9 Ci/mol), and *myo*-[2(n)-³H]inositol (sp. radioactivity 12.5 Ci/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Silica-gel GF t.l.c. plates were from Analtech, Newark, DE, U.S.A. Lipid-free bovine serum albumin, snake (*Naja naja*) venom phospholipase A₂ and all lipids used as chromatographic standards were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All solvents (reagent-grade) and inorganic salts were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A. Mouse LM fibroblasts were kindly supplied by S. Ostrove of these laboratories.

Methods

Cell culture

Macrophages were collected by peritoneal lavage in 5 ml of M199 medium containing 1% heat-inactivated pig serum, 0.1 mg of gentamicin and 20 units of heparin/ml. The cells were plated at $(5-6) \times 10^6$ per 50 mm culture dish and incubated for 2 h at 37°C in an atmosphere of CO₂/air (1:19). The non-adherent cells were removed by washing the cell sheet with five 5 ml volumes of normal saline (0.9% NaCl) using an automated cell-washing apparatus designed by P. Wightman. Mouse LM fibroblasts were routinely maintained in serum-free Higuchi medium.

Preparation of cell homogenates

Adherent macrophages (3×10^6) were scraped into 1 ml of normal saline using a rubber 'police-man' and placed in a 15 ml conical centrifuge tube. These contents were then pulse sonicated for 5 min while suspended in an ice bath. Each homogenate was then centrifuged for 10 min at 1000g and the supernatant was retained as the source of enzyme. Greater than 95% of the cells were disrupted by this procedure (Bonney *et al.*, 1979b). The protein content of this preparation was determined by the method of Lowry *et al.* (1951). Cell homogenates were routinely stored at 4°C.

Preparation of substrates

[¹⁴C]Arachidonic acid-labelled substrates were prepared from LM cells maintained in serum-free Higuchi medium containing 20 μCi of [¹⁴C]arachidonic acid per 150 cm² flask. Dual-labelled substrates were prepared from LM cells maintained in serum-free Higuchi medium devoid of choline, serine and inositol. This medium contained 20 μCi each of *myo*-[³H]inositol and [¹⁴C]arachidonic acid, [¹⁴C]choline and [³H]arachidonic acid or [¹⁴C]-ethanolamine and [³H]arachidonic acid for the preparation of dual-labelled phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine respectively. Labelled LM cells were scraped from culture flasks in methanol/water (5:2, v/v) and the lipids were extracted by the method of Bligh & Dyer (1959). Radiolabelled phospholipid components were purified on silica gel GF t.l.c. plates developed in chloroform/methanol/acetic acid/water (25:15:2:1, by vol.). Alternatively, a [¹⁴C]arachidonic acid-labelled polar lipid fraction was separated on silica gel GF t.l.c. plates developed in ethyl acetate/acetic acid (99:1, v/v) and utilized as substrate. The composition of this substrate was approx. 65% phosphatidylcholine, 20% phosphatidylethanolamine and 15% phosphatidylinositol plus phosphatidylserine. The specific radioactivities of the substrates were determined by relating lipid

phosphorus content to ³H and ¹⁴C d.p.m. Phosphorus determinations were performed by a modification of the method of Kraml (1966). These substrates were hydrolysed to >90% by phospholipase A₂ (*N. naja*) indicating the incorporation of [¹⁴C]arachidonic acid in the *sn*-2-position of the glycerol moiety. All substrates were stored at 0°C under N₂ and were stable for >60 days.

Phospholipase assay

Enzyme activities were assayed in 100 μl of 0.1 M-sodium acetate buffer, pH 4.5, containing 1 mM-EDTA, or in 0.1 M-Tris/HCl buffer, pH 8.5, containing 2 mM-Ca²⁺. This final reaction mixture included 2 μg of macrophage protein, and 10 nmol of radiolabelled substrate added as a liposomal suspension prepared by brief sonication in water. The reactions were performed at 37°C and were terminated by the addition of 3.75 vol. of methanol/chloroform (2:1, v/v). The reactants and products were extracted by the method of Bligh & Dyer (1959) and purified on silica gel GF t.l.c. plates developed in chloroform/methanol/acetic acid/water (25:15:2:1, by vol.). We have substantiated that those counts migrating with the neutral lipids in the chloroform/methanol/acetic acid/water development are fatty acid as they correspond exactly to counts associated with fatty acid standard in a light petroleum (b.p. 35–60°C)/diethyl ether/acetic acid (70:30:1, by vol.) development. These are, of course, arachidonic acid counts, as this is the only label employed. This method of extraction allowed for recovery of >90% of the reactants and products even at pH 9. Lipid components were detected by exposing them to I₂ vapour and identified by their co-migration with authentic standards. Products were quantified by scraping their corresponding spots into counting vials containing 20 ml of Aquasol II, and counting for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer.

Results

The cells on which the present study was based and from which the homogenates were made have been shown by available criteria to be mononuclear phagocytes. They are actively phagocytic, secrete lysozyme, bear Fc receptors and are strongly adherent (Bonney *et al.*, 1978).

Conditions for assay of two phospholipase A₂ activities

Initial experiments were performed to determine the optimal conditions for assaying phospholipase activities in 1000 g supernatants of macrophage homogenates. To determine the pH optima of these activities, supernatant was assayed over a range of increasing pH in the presence of 2 mM-Ca²⁺ or

1 mM-EDTA. As depicted in Fig. 1, two peaks of phospholipase activity were observed, one at pH 4.5, which does not require Ca²⁺ for activity, and another at pH 8.5, which has an absolute Ca²⁺ requirement.

To determine the optimal Ca²⁺ concentration, supernatant was assayed in the presence of increasing amounts of EDTA and Ca²⁺. These data, shown in Fig. 2., indicate that at pH 8.5 maximal activity was expressed in the presence of 2 mM-Ca²⁺ and was completely inhibited by EDTA. At pH 4.5 inhibition was exhibited by increasing Ca²⁺ concentrations and by EDTA at concentrations in excess of 1 mM.

The pH 4.5 activity was linearly dependent on the amount of protein assayed and on the duration of the assay for up to 6 h. This activity had a maximum reaction velocity of 53 nmol/h per mg of protein and was fully saturated at 100 μM substrate. The pH 8.5 activity was also linearly dependent on the amount of protein assayed and on the time of incubation for up to 24 h. It had a maximum reaction velocity of 4 nmol/h per mg of protein and was also fully saturated at 100 μM substrate. All experiments were performed within these linear ranges for both time and protein.

Fig. 1. pH optima for the hydrolysis of [¹⁴C]arachidonic acid-labelled polar lipid by resident-peritoneal-macrophage homogenate

Activities were assayed with 2 mM-CaCl₂ (●) or with 1 mM-EDTA (○) in 0.1 M-buffer. Reactions at pH 3–6 were in sodium acetate/acetic acid buffers and those at pH 7–9 were in Tris/HCl buffers. Results represent pH optima only and not absolute relative rates of the two phospholipase A₂ activities. Incubations were for 16 h. Results are reported as averages of triplicate determinations with standard deviations less than 10%.

Fig. 2. Effects of Ca^{2+} and EDTA on the pH 4.5 (○) and the pH 8.5 (●) phospholipase A_2 activities

Activities were assayed as described in the Methods section with the modifications in Ca^{2+} and EDTA concentrations as indicated. The pH 4.5 and pH 8.5 phospholipase A_2 activities were assayed for 2 h and 4 h respectively. Results are reported as averages of triplicate determinations with standard deviations less than 10%.

Identification of the products of hydrolysis

Production of non-esterified arachidonic acid from the hydrolysis of phospholipid could result from the action of a phospholipase A_2 , the successive hydrolysis of a phospholipase A_1 and a lysophospholipase, or by successive cleavages by a phospholipase C and a diacylglycerol lipase. If the enzymes are of the A_2 type, the hydrolysis of phosphatidylethanolamine or phosphatidylcholine would yield equimolar amounts of lysophospholipid and non-esterified arachidonic acid. Experiments with the dual-labelled substrates 1-acyl-2-[^3H]arachidonoylglycerophospho[^{14}C]choline, 1-acyl-2-[^3H]arachidonoylglycerophospho[^{14}C]ethanolamine and 1-acyl-2-[^{14}C]arachidonoylglycerophospho[^3H]inositol show this to be the case. The data in Fig. 3 show an equimolar recovery of arachidonic acid and lysophospholipid. The equimolar recovery of these two products establishes that the enzymes operative at both pH 4.5 and pH 8.5 are of the phospholipase A_2 type. It was observed that there was no production of [^3H]inositol, [^{14}C]diacylglycerol or [^{14}C]arachidonic acid from 1-acyl-2-[^{14}C]arachidonoylglycerophospho[^3H]inositol when assayed under these conditions (results not shown).

Determination of rates of substrate hydrolysis

Liposomes composed of a heterogeneous mixture of polar lipids more closely approximate the substrate configuration that would be in a natural

Fig. 3. Reaction products recovered from phospholipase A_2 hydrolysis

Hydrolysis products were recovered from reactions conducted as described in the Methods section using as substrates [^{14}C]ethanolamine, 1-acyl-2-[^3H]arachidonoylglycerophosphoethanolamine or 1-acyl-2-[^3H]arachidonoylglycerophospho[^{14}C]choline. The products arachidonic acid (□) and lysophospholipid (▨) were identified by t.l.c. as they co-migrated with authentic lipid standards. The pH 4.5 and pH 8.5 phospholipase A_2 activities were assayed for 2 h and 8 h respectively. Data are reported as means $\pm$ s.d. ($n = 3$).

membrane. It has been reported (Adamich *et al.*, 1979) that the preference of *N. naja* phospholipase A_2 for phosphatidylcholine over phosphatidylethanolamine can be reversed by presenting both components together in an optimally heterogeneous mixture. To determine if the macrophage enzymes exhibit differential rates of hydrolysis similar to that observed in *N. naja* phospholipase A_2 , the hydrolysis of phosphatidylethanolamine, phosphatidylcholine or phosphatidylinositol alone or combined with the polar lipids normally present in a biological membrane was measured. The polar lipid fraction was extracted from LM fibroblasts and separated from the non-polar fraction as described in the Methods section. As determined by lipid phosphorus values, polar lipid was combined with purified radiolabelled phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol at a rate of 2:1. The hydrolysis of these polar mixtures was compared with the hydrolysis of purified phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol alone. These results are shown in Fig. 4. Although the unlabelled polar lipid has sufficient phosphatidylcholine, phosphatidylethanol-

Fig. 4. Hydrolysis of [¹⁴C]arachidonate-labelled phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol in simple sonicated dispersions of the purified phospholipid (▨) or in mixed liposomes of polar lipids (□)

Simple sonicated dispersions were prepared exclusively from [¹⁴C]arachidonate-labelled phosphatidylethanolamine, phosphatidylcholine or phosphatidylinositol and assayed at a final concentration of 120 μM. Mixed liposomes were prepared from [¹⁴C]arachidonate-labelled phosphatidylethanolamine, phosphatidylcholine or phosphatidylinositol combined with a 2-fold molar excess of unlabelled polar lipid. The final concentration of the [¹⁴C]-arachidonate-labelled component in each case was 120 μM. All substrates were prepared as described in the Methods section. The pH 4.5 and pH 8.6 activities were incubated for 2 h and 8 h respectively. Data are reported as means ± S.D. (n = 3).

amine and phosphatidylinositol to compete with the radiolabelled component with which it is combined, evidence of such competition at pH 4.5 did not occur. On the contrary, significant augmentation of activity is exhibited at pH 4.5 in the hydrolysis of [³H]arachidonoyl phosphatidylethanolamine and [³H]arachidonoyl phosphatidylcholine. The pH 8.5 activity, however, hydrolyses [³H]arachidonoyl phosphatidylethanolamine and [³H]arachidonoyl phosphatidylcholine more efficiently when these substrates are presented as single purified components compared with their rates of hydrolysis when presented in combination with the unlabelled polar fraction. It can also be noted again that [¹⁴C]arachidonoyl phosphatidylinositol is not hydrolysed at either pH in the presence or in the absence of the polar mixture.

Table 1. Effect of Triton X-100 on macrophage phospholipase A₂ activities

Assays were conducted as described in the Methods section with polar substrate held constant at 100 μM and increasing amounts of Triton X-100 added. Results are reported as % of control incubations without Triton X-100 and are averages of triplicate determination with standard deviations less than 10%.

Triton X-100/substrate ratio	Phospholipase A ₂ activity (% of control)	
	pH 4.5	pH 8.5
0.5	90.3	47.7
1.0	82.8	37.7
2.0	67.7	27.6
3.0	63.1	19.3
4.0	51.5	9.8
5.0	48.5	3.8
10.0	30.2	2.5

Alteration of substrate by the inclusion of detergent

The apparent activities of many lipolytic enzymes are significantly augmented by optimal amounts of detergent. We have determined the effect of a commonly used non-ionic detergent, Triton X-100, on the phospholipase A₂ activities. We selected this detergent because it is well characterized with respect to its critical micellar concentration and optimum detergent lipid ratio for the solubilization of membranes (for review, see Helenius & Simons, 1975). Furthermore, possible detergent effects on any substrate surface charge would be minimized by using the non-ionic Triton X-100. The results in Table 1 show that at each detergent/substrate ratio employed, Triton X-100 was inhibitory to both phospholipase A₂ activities.

Discussion

Macrophages in culture synthesize and release large amounts of prostaglandins when exposed to inflammatory stimuli. To gain a greater knowledge of the mechanisms by which these cells interact with such stimuli and release these products, we sought to identify and characterize the lipolytic enzymes that may play a significant role in this sequence of events.

We have established the presence of two phospholipase A₂ activities, differing in pH optima and Ca²⁺ requirement, in homogenates of macrophages purified by adherence in culture. Both phospholipase activities have been demonstrated to be of the A₂ type by using phospholipid substrates radiolabelled in both their sn-2-fatty acid and polar-head-group moieties. We have recovered equimolar quantities of [³H]arachidonic acid and [¹⁴C]lyso-phospholipid from both phosphatidylethanolamine

and phosphatidylcholine, indicating that the hydrolysis of both phospholipids is due to the action of phospholipase A_2 .

One activity is optimal at pH 8.5 and requires 2 mM- Ca^{2+} for maximal hydrolysis. This activity can hydrolyse both phosphatidylcholine and phosphatidylethanolamine and exhibits a maximum reaction velocity of 4 nmol/h per mg of protein. This is similar to the membrane-bound enzymes characterized by other investigators in terms of pH optimum, Ca^{2+} requirement and substrate specificity (Waite & van Deenen, 1967; Nachbaur *et al.*, 1972; Ziboh & Lord, 1979). The other activity is optimal at pH 4.5 and does not have a Ca^{2+} requirement. Like the pH 8.5 activity this is inhibited by increasing Ca^{2+} concentration, a phenomenon that may be attributed to the altering of the surface charge of the substrate liposome (Franson & Waite, 1978). The pH 4.5 activity hydrolyses both phosphatidylcholine and phosphatidylethanolamine and has a maximum reaction velocity of 53 nmol/h per mg of protein. The acid pH optimum and the inhibition of activity by Ca^{2+} suggest that this enzyme is of lysosomal origin. It is similar in these respects and in its substrate specificity to the soluble lysosomal phospholipases A_2 characterized previously (Franson & Waite, 1973; Nachbaur *et al.*, 1972; Waite *et al.*, 1969; Franson *et al.*, 1972). When macrophages prelabelled with [3H]arachidonic acid are exposed to zymosan in culture, the release of [3H]prostaglandins is paralleled by a significant decrease in the counts associated with phosphatidylcholine only. We have observed that the pH 8.5 activity is augmented as the homogenate is stored at 4°C (P. D. Wightman, unpublished work). That this is due to phospholipase A_2 endogenous to possible contaminating organisms is most unlikely as all homogenates are stored in the presence of 0.01% NaN_3 . The nature of this augmentation requires further investigation therefore.

Both enzyme activities are capable of hydrolysing substrates that are unaltered by the addition of exogenous detergent. They are in fact inhibited in the hydrolysis of such altered substrate. This characteristic is in contrast with the assays for venomous and pancreatic phospholipases A_2 , which usually require that substrate phospholipids be incorporated into detergent micelles (McMurray & McGee, 1972; Nieuwenhuizen *et al.*, 1974). The observed activities are, however, subject to modulation by the nature of the substrate. At pH 8.5, purified phosphatidylethanolamine and phosphatidylcholine are hydrolysed more efficiently than when each is incorporated into liposomes composed of a mixture of other polar lipids. At pH 4.5, the reverse is true. Both phosphatidylethanolamine and phosphatidylcholine are hydrolysed most efficiently when they are presented as components in a polar

mixture. Certainly, as efforts are made to elucidate the mechanisms by which cells respond to inflammatory stimuli and release prostaglandins the relative importance of enzymes contributing to this phenomenon will be assessed. Such assessments based on the concentration of product observed in a disrupted cell assay compared with the amount of product released from an intact cell should be made with care. Indeed, the amount of product quantified in an assay using disrupted cells can be influenced as much by the nature of the substrate used as by the amount of enzyme present.

Although these phospholipases A_2 can supply amounts of non-esterified arachidonic acid in excess of that necessary to account for the 1–2 nmol of prostaglandins released/h per mg of cell protein, other lipolytic enzymes may contribute to this phenomenon. Indeed, Bell *et al.* (1979) have described a novel pathway in the human platelet leading to the production of non-esterified arachidonic acid and, ultimately, prostaglandins. Briefly, this pathway is initiated by a phospholipase C hydrolysis of phosphatidylinositol resulting in the formation of diacylglycerol (Rittenhouse-Simmons, 1979; Mauco *et al.*, 1979). Although diacylglycerol is a necessary intermediate in the synthesis of phospholipid and triacylglycerol *de novo*, Bell *et al.* (1979) propose that this molecule, rich in arachidonic acid, is hydrolysed by a diacylglycerol lipase. This hydrolysis releases arachidonic acid for utilization in the prostaglandin synthetic pathway. Alternatively, Lapetina & Cuatrecasas (1979) have shown that in the horse platelet the phospholipase C-generated diacylglycerol is rapidly phosphorylated by diacylglycerol kinase to produce phosphatidic acid. They propose that phosphatidic acid may then activate phospholipase A_2 directly or indirectly as a consequence of Ca^{2+} mobilization (Tyson *et al.*, 1976).

The liberation of arachidonic acid by mechanisms in the macrophage apart from or accessory to a direct phospholipase A_2 pathway is a matter about which we are concerned. The enzymes that function in these pathways are currently under investigation and will be included in our efforts to elucidate the manner by which the macrophage interacts with inflammatory stimuli and responds with the release of oxygenated products of arachidonic acid.

References

- Adamich, M., Roberts, M. F. & Dennis, E. A. (1979) *Biochemistry* **18**, 3308–3314
- Bell, R. L., Kennerly, D. A., Stanford, N. & Majerus, P. W. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3232–3241

- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Bonney, R. J., Wightman, P. D., Davies, P., Sadowski, S. J., Kuehl, F. A., Jr & Humes, J. L. (1978) *Biochem. J.* **176**, 433–442
- Bonney, R. J., Naruns, P., Davies, P. & Humes, J. L. (1979a) *Prostaglandins* **18**, 605–616
- Bonney, R. J., Wightman, P. D. & Davies, P. (1979b) *Biochem. Pharmacol.* **28**, 2471–2478
- Brune, K., Golatt, M., Kalin, H. & Peskar, B. A. (1978) *Nature (London)* **274**, 261–263
- Chap, H. & Douste-Blazy, L. (1974) *Eur. J. Biochem.* **48**, 351–355
- Coatt, S. & Berenholz, Y. (1973) *Annu. Rev. Biochem.* **42**, 61–90
- Franson, R., Waite, M. & Weglicki, W. (1972) *Biochemistry* **11**, 472–476
- Franson, R. C. & Waite, M. (1973) *J. Cell Biol.* **56**, 621–627
- Franson, R. & Waite, M. (1978) *Biochemistry* **17**, 4029–4033
- Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79
- Humes, J. L., Bonney, R. J., Pelus, L., Dahlgren, M. E., Sadowski, S., Kuehl, F. A., Jr. & Davies, P. (1977) *Nature (London)* **269**, 149–151
- Humes, J. L., Davies, P., Bonney, R. J. & Kuehl, F. A., Jr. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1318
- Kimze, H. & Vogt, W. (1971) *Ann. N.Y. Acad. Sci.* **180**, 123–125
- Kraml, M. (1966) *Clin. Chim. Acta* **13**, 442–448
- Lands, W. E. M. & Crawford, C. G. (1976) in *The Enzymes of Biological Membranes*, vol. 2 (Martonosi, A., ed.), pp. 3–85, Plenum Press, New York
- Lapetina, E. G. & Cuatrecasas, P. (1979) *Biochim. Biophys. Acta* **573**, 394–402
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265
- Mason, R. J., Stossel, T. P. & Vaughan, M. (1972) *J. Clin. Invest.* **51**, 2399–2407
- Mauco, G., Chap, H. & Douste-Blazy, L. (1979) *FEBS Lett.* **100**, 367–370
- McMurray, W. C. & McGee, W. L. (1972) *Annu. Rev. Biochem.* **41**, 129–160
- Nieuwenhuizen, W., Kunze, H. & deHaas, G. H. (1974) *Methods Enzymol.* **32**, 147–154
- Nachbaur, J., Colbeau, A. & Vignais, P. M. (1972) *Biochim. Biophys. Acta* **274**, 426–466
- Rittenhouse-Simmons, S. (1979) *J. Clin. Invest.* **63**, 580–587
- Stossel, T. P., Mason, R. J. & Smith, A. L. (1974) *J. Clin. Invest.* **54**, 638–645
- Tyson, C. A., Zande, H. V. & Green, D. E. (1976) *J. Biol. Chem.* **251**, 1326–1332
- van den Boosch, H. (1974) *Annu. Rev. Biochem.* **43**, 243–277
- Waite, M. & van Deenen, L. L. M. (1967) *Biochim. Biophys. Acta* **139**, 498–517
- Waite, M., Scherphof, G. L., Boshouwers, F. M. G. & van Deenen, L. L. M. (1969) *J. Lipid Res.* **10**, 411–419
- Ziboh, V. A. & Lord, J. T. (1979) *Biochem. J.* **184**, 283–290
- Zwaal, R. F. A., Roelofsen, B. & Colley, C. M. (1973) *Biochim. Biophys. Acta* **300**, 178–193